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Morphology, pollen preferences and DNA-barcoding of five Austrian species in the *Colletes succinctus* group (Hymenoptera, Apidae)

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Abstract

Most species of the *Colletes succinctus* group sensu Noskiewicz, 1936 are taxonomically uncertain. This study has chosen an integrative approach, including pollen analysis, morphology, male genitalia, morphometry, cuticle sculpture and DNA-barcoding (*CO1*) to investigate the five species that were reported from Austria. It includes a detailed analysis of the male genitalia and the first description of the *C. pannonicus* male. A syntype male from the island of Crete was designated as the lectotype of *Colletes succinctus brevigena* Noskiewicz, 1936 to fix the species identity. New distinguishing characters were found: in females the shape of the dorsal end of the fovea facialis and, in both sexes, the structure of maxillary palpi, as well as the different puncturation on the mesopleura. Unknown structures on sterna and genitalia of the males proved to be reliable morphological characters. An identification key is provided for all studied species. Morphometry of females did not allow a clear distinction of species. *CO1* sequencing confirmed previous studies that only *C. collaris* clearly deviates from the other species, including *C. pannonicus* that was analysed for the first time. Pollen analysis showed polylectic, as well as oligolectic, pollen-collecting behaviour. The collected pollen of *C. pannonicus* confirmed the field observations that this species is strictly oligolectic on *Tripolium pannonicum*. Due to pronounced intraspecific variation, it is assumed that the species of the *C. succinctus* group are either species in *statu nascendi* or very young species. Therefore, it remains important to include ecological data in species identification.

Key Words

Bee, identification, integrative taxonomy, morphometrics, phylogeny, *Colletes hederae*, *Colletes brevigena*, *Colletes collaris*, *Colletes pannonicus*, *Colletes halophilus*

Introduction

Colletes Latreille, 1802 is a solitary bee genus belonging to the family Colletidae. Their common English name "polyester bees" is derived from a characteristic cellophane nest lining. The female produces a polyester secretion in the abdominal Dufour gland (Albans et al. 1980) and uses it to coat the nest cell walls for waterproofing with their widely split tongue (Westrich 1989).

The genus comprises 522 described species (Proshchalykin and Kuhlmann 2018; Kuhlmann and Smit 2018; Kuhlmann 2019) which are distributed all around the world, except Australia, Antarctica, Madagascar and parts of Southeast Asia (Michener 2007; Kuhlmann 2014). Only 21 species of the genus *Colletes* are reported from Austria (Gusenleitner et al. 2012). Based on morphological characters, the Palaearctic species were divided into 26 species groups (Noskiewicz 1936). Especially the *Colletes succinctus* group is notorious for being a taxonomic challenge. It is defined by two synapomorphies: two deep lateral pits at sternum 6 (subgenital plate) of the males and a red-brown transparent basal margin of gaster tergum 1 (Noskiewicz 1936, Kuhlmann et al. 2007).

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In his detailed revision of Palaearctic species, Noskiewicz (1936) assigned only two species, *Colletes succinctus* (Linnaeus, 1758) and *C. collaris* Dours, 1872, and two newly-described subspecies (*C. succinctus aegyptiacus* and *C. succinctus brevigena*) to the *C. succinctus* group. Today, this group comprises 14 species, some of them occurring in temperate Asia and North Africa and seven being native to Europe (Kuhlmann 2000, 2003; Hölzler and Mazzucco 2011): *Colletes succinctus*, *C. collaris*, *C. brevigena* Noskiewicz, 1936 (now in the rank of species), *C. halophilus* Verhoeff, 1944, *C. hederae* Schmidt & Westrich, 1993, *C. standfussi* Kuhlmann, 2003 and *C. pannonicus* Hölzler & Mazzucco, 2011. Although the species have different distribution areas in the Palaearctic, all except *C. halophilus* (from North Sea coastal habitats; Westrich 1989; Kuhlmann et al. 2007) and *C. standfussi* (from Greece; Kuhlmann 2003) occur sympatrically in the eastern parts of Austria (Gusenleitner et al. 2012) (Table 1).

According to collection and literature data, the Austrian species of the *C. succinctus* group have one generation per year (monovoltine). Although they are all "late-summer bees" (Scheuchl and Willner 2016), collection data suggest that they differ in their phenology. *Colletes succinctus* emerges first early to mid-August (Scheuchl and Willner 2016), followed by *C. brevigena* and *C. collaris* in late August (Westrich 1997; Zettel et al. 2006; Standfuss 2009). The holotype of *C. pannonicus* was collected mid-September (Hölzler and Mazzucco 2011) and *C. hederae* seems to be the species that is most adapted to the cool season. It can be found from late August until November (Kuhlmann et al. 2007).

Regarding the provisions for their offspring, the Austrian species of this group reportedly show different pollen preferences: the species are described as either polylectic, oligolectic or pseudo-oligolectic (Bischoff et al. 2005; Müller and Kuhlmann 2008; Westrich 2008; Teppner and Brosch 2015). *Colletes succinctus* and *C. brevigena* show polylectic behaviour (Michener 2007; Müller and Kuhlmann 2008). However, it should be mentioned that *C. succinctus*, despite being a generalist, prefers heather (*Calluna* sp.) which leads to its vernacular name "heather bee". *Colletes collaris* and *C. halophilus* are typical oligolectic bees, preferring pollen of Asteraceae (Westrich 1997; Kuhlmann et al. 2007; Müller and Kuhlmann 2008) and the term pseudo-oligolectic was used for *C. hederae* (Teppner and Brosch 2015). As its common name suggests, the "ivy bee" shows a strong preference for ivy (*Hedera* sp.) (Schmidt and Westrich 1993) and is widespread throughout Europe, with only a few gaps in Scandinavia (Rathjen 1998). Nonetheless, it also collects pollen from other flowers before the ivy starts blooming (Müller and Kuhlmann 2008). *Colletes hederae* was originally a Mediterranean species, but is currently spreading to Central and Western Europe at a rapid rate (Schmid-Egger 1997; Rathjen 1998; Vereecken et al. 2009, Saure et al. 2019). Additionally, in Austria, it shows a rapid expansion (Neumayer 2012; Zettel and Wiesbauer 2014; Ebmer et al. 2018). Due to its strong similarity to *C. succinctus* and *C. halophilus*, *C. hederae* was described recently, although specimens of the genus *Colletes* collecting pollen on ivy have been reported for a long time (Richards 1979; Janvier 1979, 1980; Westrich 1989). As the latest addition to the species group, *Colletes pannonicus* was described from a population using the pollen of *Tripolium pannonicum* (sea aster), supposedly being oligolectic on Asteraceae (Hölzler and Mazzucco 2011).

Cladograms, based on molecular data, show strong agreement with Noskiewicz's (1936) morphologically based species groups (Kuhlmann et al. 2009). However, the mitochondrial gene fragment cytochrome oxidase 1 (*CO1*) did not show any species-specific, fixed differences within the *Colletes succinctus* group (Kuhlmann et al. 2007, 2009; Magnacca and Brown 2012; Dellicour et al. 2014). Misinterpretations of taxa may have led to unreliable analyses and a phylogenetic analysis, including *C. pannonicus*, has not yet been performed.

The taxonomy and phylogeny of the species of the *C. succinctus* group have been the object of recent discussions and investigations (e.g. Kuhlmann et al. 2007; Müller and Kuhlmann 2008; Kuhlmann et al. 2009; Magnacca and Brown 2012; Dellicour et al. 2014). The original descriptions of species described after Noskiewicz's (1936) revision compare the new taxa only with one previously described (usually sympatric) species, thereby neglecting similar species from other areas. However, with the exceptions of *C. collaris* and *C. standfussi*, which both can be easily recognised, the species are morphologically very difficult to distinguish (Kuhlmann 2003). Consequently, their different phenology, their pollen preferences, as well as their habitat preferences, were used for differentiation (e.g. Verhoeff 1944; Schmidt and Westrich 1993; Kuhlmann 2003; Kuhlmann et al. 2007; Hölzler and Mazzucco 2011).

The aim of this study is to compare European species of the *C. succinctus* group, with focus on the five species that were previously reported from eastern Austria. Therefore,

Table 1. Distribution of the European species of the *Colletes succinctus* group. Austrian taxa treated in bold.

Species	Distribution
C. brevigena	Europe and Mediterranean, eastwards to Iran, southwards to North Africa, Egypt and Tunisia (Ascher and Pickering 2011–2018).
C. collaris	Widespread in the Palaearctic, temperate Asia except for Southwest Asia; in Europe documented for Central Europe, France and Spain (Westrich 1997, Ascher and Pickering 2020).
C. halophilus	Coastal habitats of the North Sea (Westrich 1989, Kuhlmann et al. 2007, Ascher and Pickering 2011–2018).
C. hederae	Currently spreading from the Mediterranean of Europe to Central and Western Europe, reported from Great Britain, Spain, Italy, Croatia, Greece (Schmid-Egger 1997, Rathjen 1998, Vereecken et al. 2009).
C. intricans	North Africa, Iberian Peninsula (Ascher and Pickering 2020).
C. pannonicus	Endemic to the area around Lake Neusiedl, Austria (Hölzler and Mazzucco 2011).
C. standfussi	Endemic to Thessaly, Greece (Kuhlmann 2003).
C. succinctus	Southern and central Europe, in Great Britain, in the north as far as Finland and Sweden, in western Asia as far as Kazakhstan (Westrich 1989, Kuhlmann et al. 2007, Ascher and Pickering 2020).

an integrative approach was designed, including morphology, statistical analyses of morphometrics, pollen analyses, as well as DNA-barcoding, to exclude the possibility of misidentification in previously-studied material and to provide comparison sequences for future investigations.

Material and methods

Specimens and preparations

The study included 270 specimens (Suppl. material 1: Appendix 1) which were either newly collected or provided by the Natural History Museum of Vienna (NHM), the Upper Austrian State Museum and private collections. Pin-mounted specimens were obtained from collections. Fresh bees were collected from July to September 2017 in Vienna, Lower Austria (Retz and Ollersdorf), Upper Austria (Linz) and Burgenland around Lake Neusiedl, as well as in Poland (around the city of Sierakow). Collecting was carried out with an insect net and the bees were either euthanised in 96% ethanol to preserve DNA for DNA-barcoding or in a vial with ethyl acetate vapour.

Morphological studies

To find additional, previously-unknown morphological characters for species distinction, both females and males were examined by light microscopy and compared between species. Since a description of the males of *C. pannonicus* has not yet been published, special attention was paid to this species and a detailed description is given in this work. Much attention was given to the proboscis of the females and to sterna 6–8 and genitalia of the males. Therefore, these body parts were manually dissected. Morphological descriptions were chiefly based on the terminology of Michener (2007) and Boudinot (2013). However, in order to get a better overview of the male's structures of sterna 6–8 and genitalia, specific terms were additionally introduced.

For illustration of the species-specific differences, photography, as well as scanning electron microscopy, was used:

Stacked digital images of the different parts of sterna 6–8 and genitalia were acquired with a Leica DFC490 camera attached to a Leica Z16 APO zoom microscope, using Leica Application Suite 4.10.0 software. Afterwards, the digital images were stacked with ZerenaStacker 64-bit and processed with Adobe Photoshop 7.0. After illustration of the entire genital capsule, further dissection became necessary to see all important structures: a median cut between the two valvulae was performed, followed by removing the valvulae from the gonostyli. These parts were also illustrated by photography.

To illustrate different structures of proboscis and head of females, a scanning electron microscope (Philips XL 30 ESEM) was used. After dissection, the samples were washed for dehydration three times in 100% ethanol and three times in 100% acetone for 15 minutes each. For drying, the critical point dryer (LEICA EM MED020) was used for around 1.56 minutes with the settings: velocity – medium; delude time – 120 seconds; exchange steps – 5; cycles – 18; heating process – slow and speed – slow. Afterwards, the dried proboscides were glued to a copper foil with conductive silver and mounted on a carbon-taped stub. The dried heads were glued directly to the stub. For gold coating, a sputter coater (LEICA EM CPD300) was used for around 120 seconds. Images were taken with the scanning electron microscope and the programme Scandium 5.1 was used to add the scale bar.

Morphometry

In total, 103 females were used to obtain morphometric data for analysis (Suppl. material 2: Appendix 2): 10 specimens of *C. halophilus*, 11 specimens of *C. pannonicus*, 14 specimens of *C. brevigena*, 20 specimens of *C. collaris*, 22 specimens of *C. hederae* and 26 specimens of *C. succinctus*. The following distances, measured on the head and between the tegulae, were investigated (Figs 1, 2):

head length (HL) – maximum head length, measured in exact frontal view along the mid-line, from vertex to distal margin of clypeus;
head width (HW) – maximum head width, measured in exact frontal view from one outer edge of the compound eye to the other;
eye length (EL) – length of the eye, measured in latero-frontal view from the most dorsal point to the most ventral point of the left compound eye;

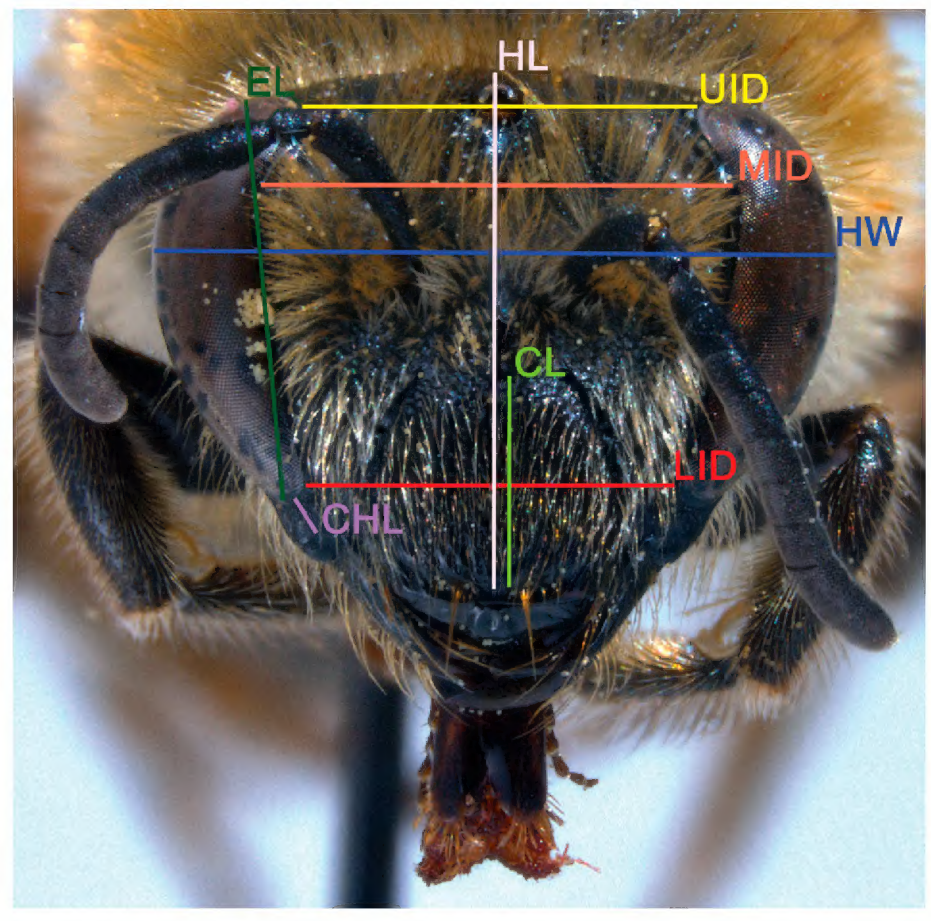

Figure 1. Frontal Measuring distances for a female of the *Colletes succinctus* group. **HL** – head length, **HW** – head width, **EL** – eye length, **UID** – upper interocular distance, **LID** – lower interocular distance, **MID** – middle interocular distance, **CL** – clypeus length, **CHL** – cheek length.

Figure 2. Dorsal measuring distance for a female of the *Colletes succinctus* group. **TW** – thorax width.

upper interocular distance (UID) – shortest distance between the dorsal margins of the compound eyes, measured in dorsal view;
lower interocular distance (LID) – shortest distance between the ventral margins of the compound eyes, measured in frontal view;
median interocular distance (MID) – longest distance between the inner margins of the compound eyes, measured in frontal view;
clypeus length (CL) – maximum clypeus length, measured in frontal view from the anterior to the posterior margin;
cheek length (CHL) – minimum length of the gena, measured in latero-frontal view from lower eye margin to mandible;
thorax width (TW) – maximum distance between the two mesal edges of the tegulae, measured in dorsal view.

Due to their very similar morphological characters *C. brevigena* and *C. pannonicus* were examined in more detail. As already done in the species description of Hölzler and Mazzucco (2011), the relationship of head width (HW) and thorax width (TW) for *C. brevigena* and *C. pannonicus* was calculated in form of an index:

$$\text{Head-thorax index} = TW / HW \times 100$$

Measurements were conducted at different magnifications (24–76.8×) using a calibrated LEICA MZ6 binocular microscope with an ocular micrometre and later converted to millimetres. The statistics programme PAST3 (Hammer et al. 2001) was used to conduct principal component analysis (PCA) and discriminant analysis (LDA). The logarithmic morphometric values were used for all analyses. The PCA was based on a correlation matrix and 95% confidence intervals of variances were calculated using 1,000 Bootstrap re-samplings. For LDA, the specimens were assigned to six hypothetical groups, based on their morphological characters (*C. succinctus*, *C. collaris*, *C. brevigena*, *C. halophilus*, *C. hederae* and *C. pannonicus*) and the Jackknife method was used for re-sampling. Due to different morphological characters, the examined species *C. brevigena* was divided into two groups (Austrian and Mediterranean specimens) and analysed separately by LDA.

Pollen analyses

For pollen analyses, the pollen loads of 32 fresh and 41 dried female specimens (n = 73) were examined (Suppl. material 3: Appendix 3). The filling ratio of the pollen loads was determined between the grades one and five (see Müller and Kuhlmann 2008): "Five" defined fully-loaded hind legs and propodeum, so that no hair was visible and "one" meant that only one fifth of the collecting structures was loaded. Filling ratios in between (2–4) were estimated according to the researcher's personal assessment. Furthermore, these filling ratios were used to calculate the correlation between the different filling loads and the amount of pollen types. Therefore, the Pearson correlation coefficient was calculated using Past3 (Hammer et al. 2001). The pollen was removed with a fine needle and placed on a glass object slide. After prevention of clotting accumulation by adding a drop of 85% ethanol, the pollen grains were fixed and dyed with a mixture of Kaisers Glycerine-Gelatine (ROTH) and alkaline Fuchsine. Afterwards, up to 300 pollen grains per slide were counted by using a NIKON ECLIPSE E800 light microscope.

For pollen determination, literature (Beug 2004, Hesse et al. 2009), as well as the databases paldat, ponet and pollen.tstebler, were used. Illustrations were made with the programme NIS-Elements D (4.51.01) and edited with Adobe Photoshop 7.0. Furthermore, the percentage for each pollen type was calculated and all types below 5% were identified as impurities.

DNA-barcoding

The legs of 46 specimens were used for DNA barcoding (Suppl. material 4: Appendix 4). In preparation for DNA extraction, the ethanol, in which some of the animals were stored, had to be washed out with PBS (phosphate buffered saline). Afterwards, the samples were shock-frozen in a mixture of dry ice and ethanol and crushed. The following DNA extraction was performed using Qiagen's "DNeasy Blood and Tissue Kit" and the protocol "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)".

After extraction, the samples were analysed with a nanodrop (Nanodrop 200/2000c Spectrophotometer) for DNA quantification and only samples containing sufficient DNA were used for further analysis. For Polymerase Chain Reaction (PCR) 25 µl MM Biozym Red HS Taq Master Mix, 21 µl molecular grade water (Sigma

Aldrich) as well as 1.5 µl of each primer and the respective DNA sample (1–2 µl) were mixed. The PCR machine "TProfessional Thermocycler" (Biometra) was used to amplify the gene using the primer-pair LCO1490 and HCO2198 (Folmer et al. 1994) in the following settings: the starting denaturation cycle at 95 °C for 3 minutes was followed by 33 cycles for 30 seconds each, 33 alignment cycles at 47 °C for 30 seconds each, 33 elongation cycles at 72 °C for 45 seconds each and one final cycle at 72 °C for 10 minutes.

For the subsequent gel electrophoresis, a mixture of 1× TBE buffer and agarose gel (1% w/v) was used. In total, 3 µl of the samples, together with 1.5 µl Orange DNA Loading Dye (Thermo Science) and the Sizemarker GeneRuler 1kb – DNA Ladder (Thermo Science), were loaded on to the gel.

The purification was carried out with Qiagen's "QIAquick PCR Purification Kit" and the DNA sequencing was executed by Microsynth AG.

Unfortunately, only 21 samples were sequenced successfully: 20 specimens of the European *C. succinctus* group and one specimen of *C. creticus*, which was used as an outgroup (Suppl. material 4: Appendix 4). The remaining samples could not be used for further analyses due to either contamination or unsuccessful DNA extraction. Furthermore, some sequences could not be used because of infestation with *Wolbachia* (a genus of gram-negative bacteria common in sexual organs of arthropods) and very poor electropherograms which could not be evaluated. In general, electropherograms were not ideal, which is probably due to an artefact band (200 bp). Therefore, base-calling in all electropherograms had to be checked carefully.

The obtained electropherograms were proof-read, aligned and cut to the same length by removing the primer sequences with Bioedit 7.2.6. and FinchTV 1.4.0 (Geospiza, Inc). POPArt (Bandelt et al. 1999) was used to create a median-joining network from the sequences obtained, as well as from reference data (47 sequences) from the Barcode of Life Database (BOLD) (Ratnasingham and Hebert 2007) (Suppl. material 5: Appendix 5). In addition, genetic distances were calculated and a neighbour-joining tree was generated with MEGA 7.0.26 (Kumar et al. 2016) before it was illustrated with FigTree 1.4.3.

Results

Morphology of the species

In addition to already-known morphological features, new characters were discovered to better distinguish between the Austrian species of the *Colletes succinctus* group (Table 2, Fig. 3).

Table 2. Expression of the distinctive characters (females and males) of the investigated species in the *C. succinctus* group. Newly found characters for species differentiation, identified in this study in bold font and already known characters extracted from literature (Noskiewicz 1936; Schmidt and Westrich 1993; Burger 2010; Hölzler and Mazzucco 2011).

	Colletes succinctus	*Colletes collaris*	*Colletes brevigena*	*Colletes hederae*	*Colletes pannonicus*
shape of the dorsal end of fovea facialis	**dorsally extended, with oval-shaped margin at the dorsal end**	**dorsally extended, narrower than in *C. succinctus*, with slightly pointed apex**	**widening towards dorsal end, tapering to a medio-lateral point, lateral margin more depressed than mesal margin**	**widening towards dorsal end, rounded dorsal margin**	**widening towards dorsal end, with deep, broad and straight dorsal margin**
puncturation on frons	**cuticle smooth between puncturation**	**cuticle reticulated between puncturation**	**cuticle smooth between puncturation**	**cuticle smooth between puncturation**	**cuticle smooth between puncturation**
supra-clypeus	**with smooth centre, variable in size**	**with smooth centre, variable in size**	dull, with punctures of the same size as on clypeus	**with smooth centre, variable in size**	shiny, with larger punctures than on clypeus
clypeus	densely and coarsely punctured with lateral longitudinal wrinkles, slightly inclining inwards before the basal end, with transverse furrow at the lower end	coarsely punctured, no transverse furrow at the lower end, with longitudinal wrinkles, **inclining mesad towards the end**	longitudinally wrinkled, distal margin of clypeus exceeding mandible base	distally with inwardly inclined, longitudinal wrinkles	distal margin of clypeus not exceeding mandible base, **longitudinal wrinkles, latero-distally slightly inclined mesad**
galea	**shiny,** microstructure-free between sensilla	dull and reticulated between sensilla	dull and reticulated part between sensilla restricted to the distal half	dull and reticulated between sensilla	dull and reticulated between sensilla
maxillary palpi	**segments short and stout**	segments long and lean	segments long and lean	segments long and lean	segments long and lean
mesonotum	strongly punctured, with shiny centre	coarsely punctured, with black-brown hairs in its centre	densely punctured	more finely punctured than in *C. brevigena*, **with a shiny centre variable in its size**	**densely punctured, with a shiny centre variable in its size**
mesopleura	puncturation with distances at most the diameter of a puncture, usually smaller	puncturation with shiny intervals that are larger than diameters of punctures	densely punctured, (sporadically) punctures can merge and form wrinkles	densely punctured, punctures merge and form wrinkles	densely punctured, punctures can merge and form wrinkles
propodeum	hairy	**hairless centre of declivity**	hairy	hairy	hairy
setae on terga	broad stripes of setae at posterior margins	**narrow stripes of setae at posterior margins**	broad stripes of setae at posterior margins	broad stripes of setae at posterior margins	broad stripes of setae at posterior margins
tergum 1	finely and densely punctured, **with distances of punctures as long as a diameter of puncture**	more deeply and coarsely punctured than in *C. succinctus*	more coarsely, densely and finely punctured than in *C. succinctus* (punctures with very short distances in between)	more finely punctured than in other species, less densely punctured than in *C. halophilus*	more finely and densely punctured than in *C. brevigena*, **(sporadically) distance of punctures as long as a diameter of puncture**

Figure 3. Important morphological characters for species differentiation of the Austrian species of the *Colletes succinctus* group: (**A–C**) Female *C. succinctus*: **A.** Fovea facialis; **B.** Terga 1 and 2; **C.** Proboscis; (**D–F**) Female: *C. collaris*: **D.** Fovea facialis; **E.** Terga 1 and 2; **F.** Cuticle of frons; (**G–I**) Female *C. brevigena*: **G.** Fovea facialis; **H.** Terga 1 and 2; **I.** Cuticle of mesopleura; (**J–L**) Female *C. hederae*: **J.** Fovea facialis; **K.** Terga 1 and 2; **L.** Proboscis; (**M–O**) Female *C. pannonicus*: **M.** Fovea facialis; **N.** Terga 1 and 2; **O.** Clypeus. **ant** – antenna, **ce** – complex eye, **fov** – fovea facialis, **oc** – lateral ocellus, **mxp** – maxillary palpus, **T1** – tergum 1, **T2** – tergum 2.

Morphological characters of male sterna 6–8 and genitalia

In addition to external morphological characters, the male specimens can also be distinguished by shape and pubescence of sterna 6–8 and the morphology of the genitalia. It was possible to find differences in their shape and pubescence.

General description, *Colletes succinctus* group:

Sternum 6: large, lacking lateral tubercles; the convex hind margin medially protruded and with variably-developed blunt corners at each side. Before hind margin with two deep lateral grooves (**lgr**). An oval-shaped translucent area (**ota**) of variable size in the middle.

Sternum 7: base (**bs**) curved, narrow, connected with distal structures via a bridge (**br**) with a narrow sclerotised medial stalk. Distal part strongly modified: a central, diamond-shaped, distally bifid medial elevation (**mel**) leads to strongly sclerotised basal shoulders (**sh**), which bear the paired wings (**wg**). Except for *C. collaris* (see description of *C. collaris*), each wing consisting of a sclerotised, densely pilose medial processes (**mp**) and a weakly sclerotised, flexible lateral part with a hairy basal arm (**ba**) and a distal, almost bold membrane (**mbr**).

Sternum 8: sub-rhomboidal. Anterior spiculum (**spi**) strongly elongated. Lateral processes (**lpr**) bifid, bearing muscle attachments. Distal process (**dpr**) curved ventrally, with dense tuft of long setae on dorsal margin.

Genital capsule: stout, most parts, including gonobase, heavily sclerotised. Gonopod (**gpo**) smooth and hairless, dorsolaterally with oblique depression; ventrally fused with gonostylus, dorsally separated from it by a deep fissure. Gonostylus (**gst**) curved ventrally; mesoventrally with a ridge bearing a row of setae (**rs**), distally with a hairy, medially curved gonostylus membrane (**gme**) of approximately triangular shape. Volsella (**vol**) strongly developed; basivolsella (**bvo**) short; digitus (**dig**) and cuspis (**cus**) both plate-shaped, their opposing surfaces with numerous stout, short teeth. Penisvalva (**val**) with slender base; distal part curved ventrally, with several modifications: a heavily-sclerotised mesodorsal ridge (**mdr**), a laterodorsal membrane (**ldm**), a basolateral groove (**blg**) often surrounded by stout spines, and a lateral area (**lar**) bearing numerous, often spine-like setae.

Compared to other species groups, the distal part of sternum 7 is smaller and distinctly shorter in the *C. succinctus* group (Noskiewicz 1936). The specific structures, described above, cannot be clearly homologised with the structures of other species groups.

Specific characters, *C. collaris*:

Sternum 6: Stout, lateral edges extended to posterior, appears long and broad. Little hair on the disc, blunt corners weakly developed. Lateral grooves (**lgr**) small and spherical (Fig. 4A). – Sternum 7: Short, transversely oval paired wings (**wg**) with densely hairy basal arm (**bs**), without membrane and medial process. Strongly developed shoulders (**sh**) (Fig. 4B). – Sternum 8: Strongly sclerotised median process of the anterior spiculum (**spi**) reaching the posterior apex, with short bifurcation in its centre. Distal process (**dpr**) sitting on small outwardly curved shoulders (Fig. 4C, D). – Genital capsule: Prominent volsella (**vol**) with slender digitus (Fig. 5B, D).

Specific characters, *C. succinctus*:

Sternum 6: Prominent inwardly inclined lateral grooves (**lgr**), long and oval shaped. Weakly pronounced blunt corners on convex hind margin and oval-shaped translucent area (**ota**), 1.5–2 times larger than lateral grooves (**lgr**) (Fig. 6A). – Sternum 7: Wide in appearance, with strong shoulders (**sh**). Basal arm (**bs**) of the wing (**wg**) with setae, especially in its proximal half. Horizontally directed distal margin of membrane (**mbr**) with long setae (Fig. 6B). – Sternum 8: Distal process (**dpr**) basal with wide outwardly curved shoulders. Sclerotised anterior spiculum (**spi**) with strong central bifurcation (Fig. 6C, D). – Genital capsule: No species-specific characters were detected (Fig. 7).

Specific characters, *C. halophilus*:

Sternum 6: Prominent oval-shaped translucent area with hairless centre (**ota**) (Fig. 8A). – Sternum 7: Hind margins of membrane (**mbr**) of distal processes of sternum 7 (**wg**) almost straight (Fig. 8B). – Sternum 8: Distal process (**dpr**) basal with outwardly curved shoulders. Weakly pronounced bifurcation of median spiculum (**spi**) (Fig. 8C, D). – Genital capsule: In lateral view, membrane of gonostylus (**gme**) of genital arched dorsally, with dorsobasal knob and densely hairy (Fig. 9D).

Specific characters, *C. hederae*:

Sternum 7: Basal arm (**bs**) narrow at base and widening distally, densely hairy. Membrane (**mbr**) convexly curved, proximal part with dense setae, short setae at distal edge. Medial bifid elevation (**mel**) strongly pronounced (Fig. 10B). – Sterna 6, 8 and genital capsule: no species-specific characters were detected (Figs 10A, C, D, 11).

For species-specific characters of the male terminal structures and genitalia of *C. brevigena*, see the next chapter.

Lectotype designation of *C. brevigena*

Noskiewicz (1936) described *C. succinctus* ssp. *brevigena*, based on specimens from a large distribution area:

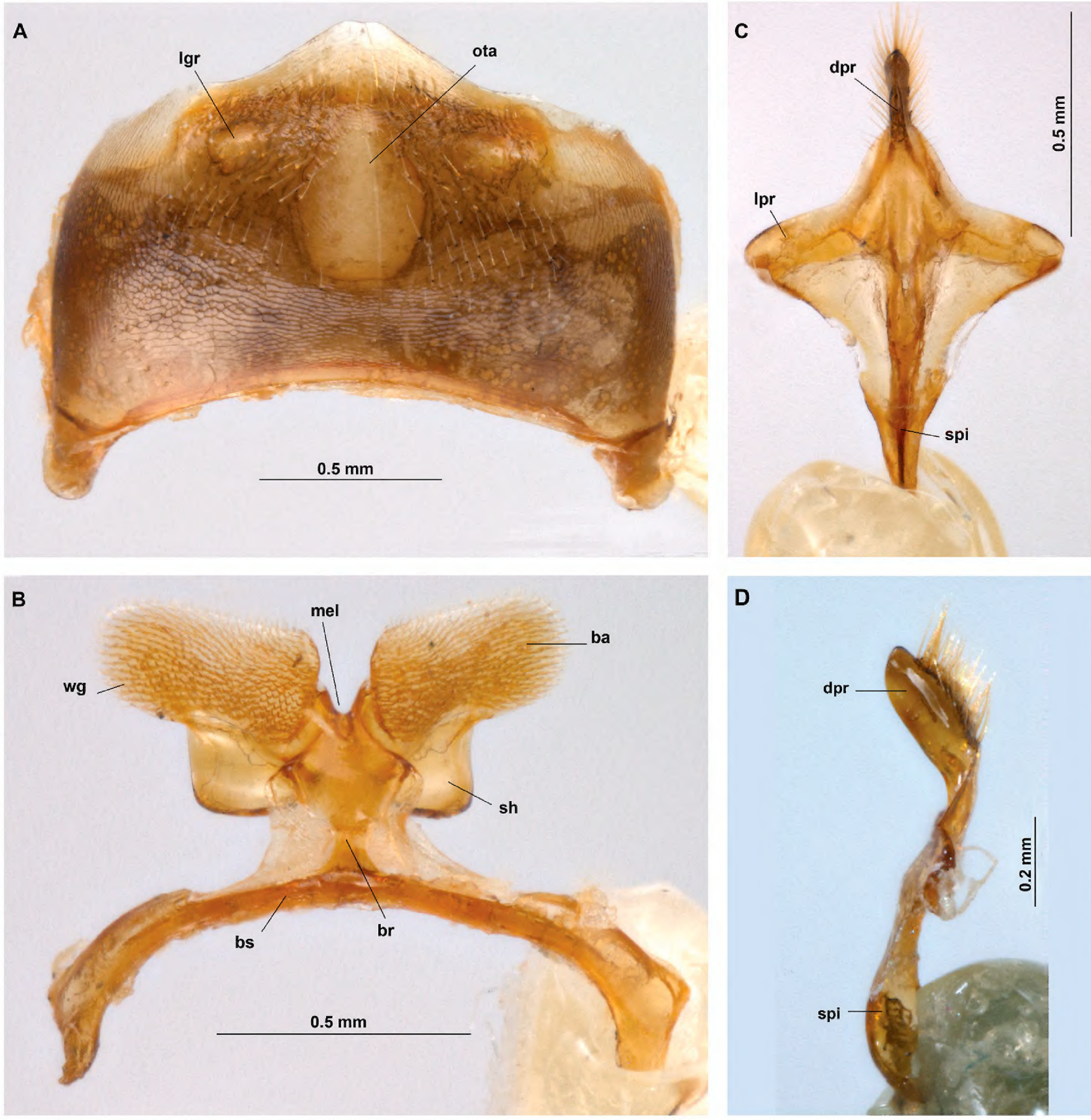

Figure 4. Male terminal structures of *Colletes collaris*, specimen no. 16 (LM): **A.** Ventral view of sternum 6; **B.** Ventral view of sternum 7; **C.** Ventral view of sternum 8; **D.** Lateral view of sternum 8. **ba** – basal arm, **br** – bridge, **bs** – base, **dpr** – distal process, **lgr** – lateral grooves, **lpr** – lateral process, **mbr** – membrane, **mel** – median elevation, **mp** – median process, **ota** – oval-shaped translucent area, **sh** – shoulder, **spi** – spiculum, **wg** – wing.

Macedonia, Dalmatia, Cyprus, Crete, North Persia and the Caucasus; however, he questioned the synsubspecificity of a male from Austria near Neusiedlersee (note that this is the type area of *C. pannonicus*). Noskiewicz (1936) did not designate a holotype; a lectotype was not selected by subsequent authors. The depositories of the syntype series were not published by Noskiewicz (1936), but later it was reported that no types of *C. brevigena* are represented in the Noskiewicz collection in the Museum of Natural History, University of Wroclaw, Poland (Wanat et al. 2014; and Marek Wanat, pers. comm. to Herbert Zettel).

The Natural History Museum Vienna keeps eleven male specimens, all identically labelled "Colletes ♂ succinctus L. ssp. brevigena Nosk. det. Noskiewicz.", which were studied by Noskiewicz in the course of preparing his monograph and, therefore, are putative syntypes. For the reason of taxonomic stability, we select a male (no. 270) from Crete (Greece) as the lectotype (see Figs 12–14). Arguments for selecting this specimen were (1) its almost perfect condition and (2) avoidance of a type locality in the southern Caucasus which would make future molecular research on this taxon more difficult.

Lectotype (♂, present designation, Natural History Museum Vienna): "Oertzen Creta 1884." [= Crete Island, Greece; printed], "Colletes ♂ succinctus L. ssp. brevigena Nosk. det. Noskiewicz." [mostly handwritten], "270" [handwritten], "Colletes brevigena Noskiewicz, 1936 det.

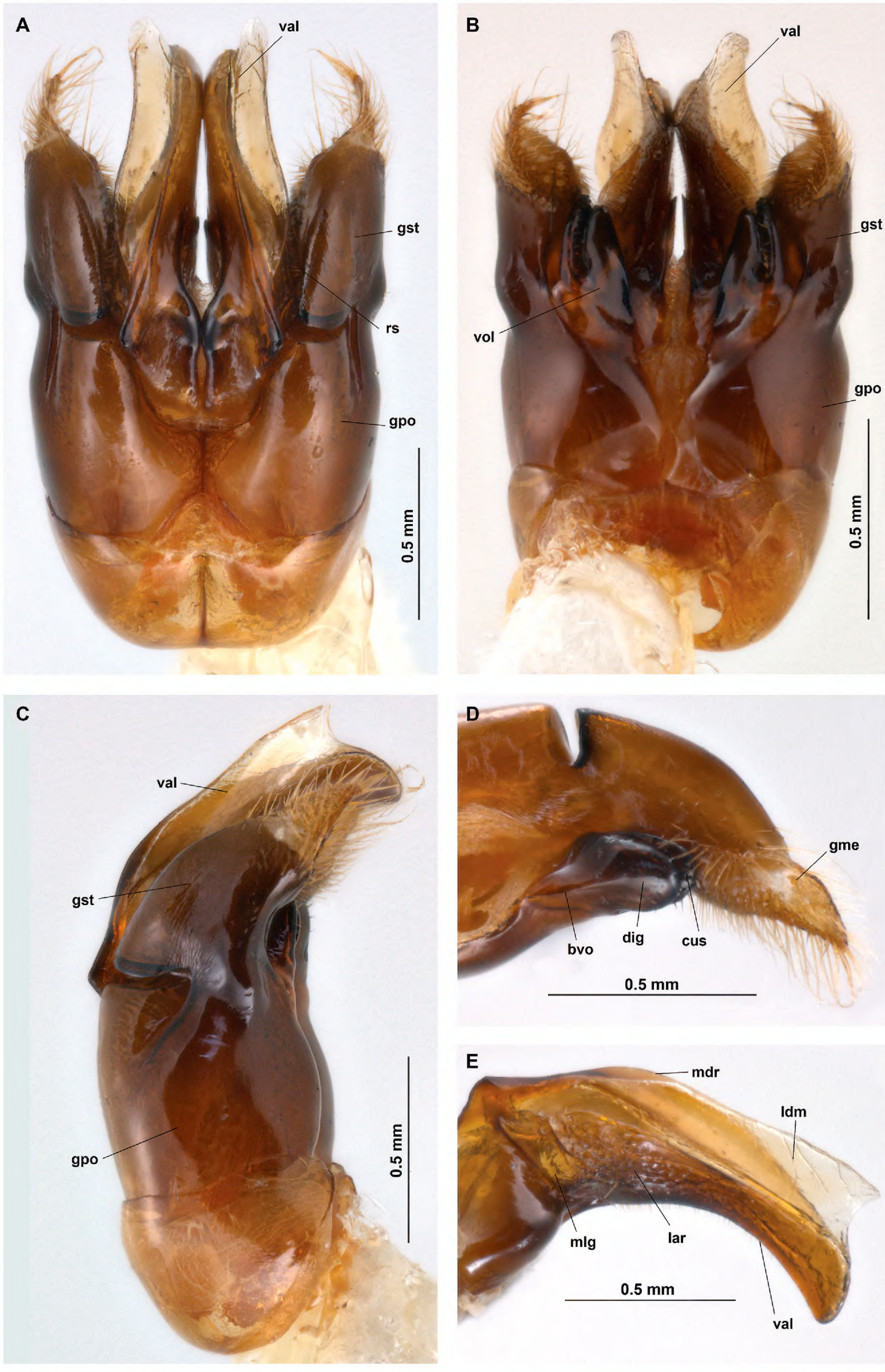

Figure 5. Male genitalia of *Colletes collaris*, specimen no. 16 (LM): **A.** Dorsal view of genital capsule; **B.** Ventral view of genital capsule; **C.** Lateral view of genital capsule; **D.** Mediolateral view of gonopod and gonostylus with volsella; **E.** Lateral view of penis valva. **blg** – basolateral groove, **bvo** – basivolsella, **us** – cuspis, **dig** – digitus, **gme** – gonostylus membrane, **gpo** – gonopod, **gst** – gonostylus, **lar** – lateral area, **ldm** – laterodorsal membrane, **mdr** – mesodorsal ridge, **rs** – row of setae, **val** – penis valva, **vol** – volsella.

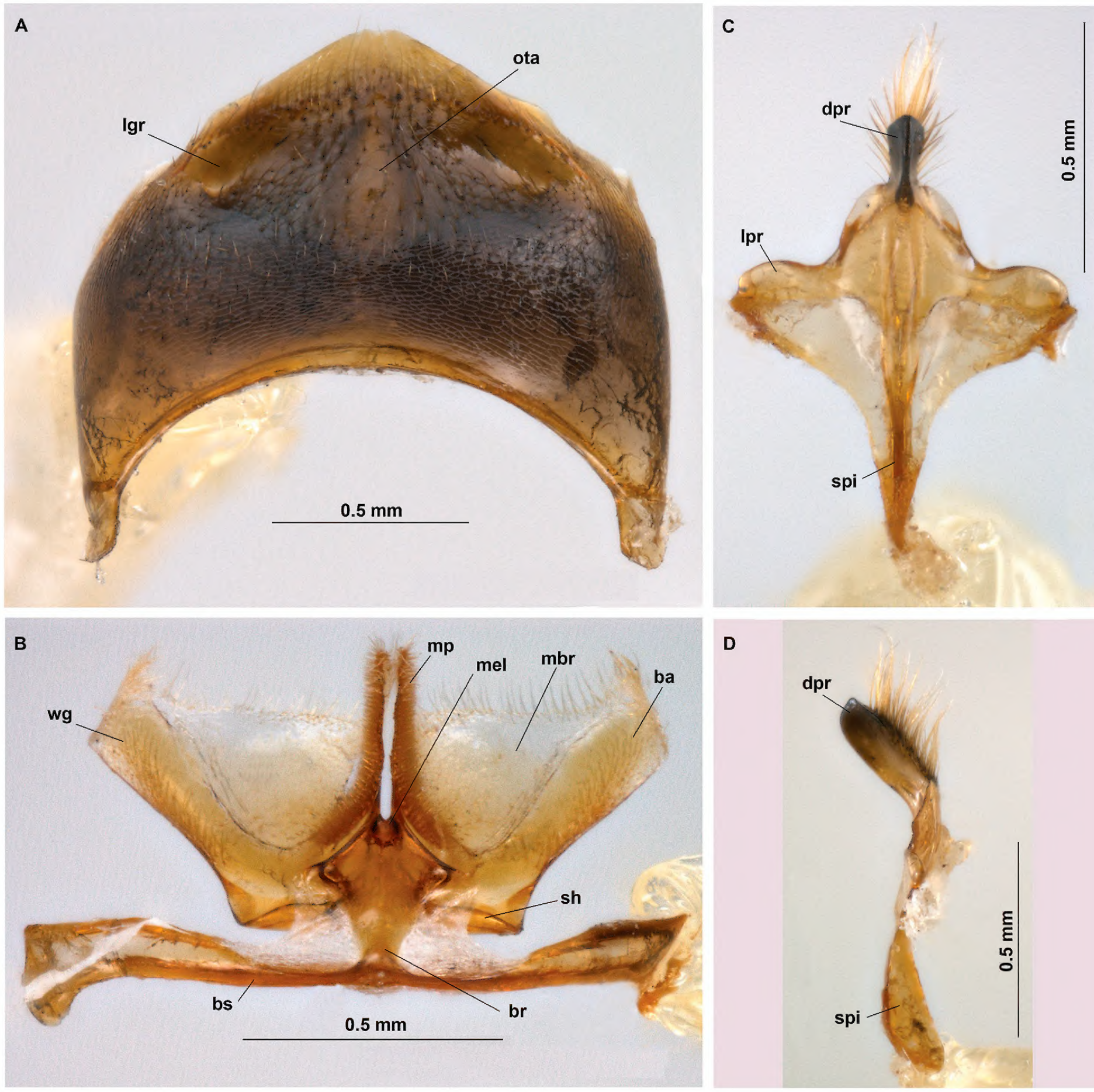

Figure 6. Male terminal structures of *Colletes succinctus*, specimen no. 243 (LM): **A.** Ventral view of sternum 6; **B.** Ventral view of sternum 7; **C.** Ventral view of sternum 8; **D.** Lateral view of sternum 8. **ba** – basal arm, **br** – bridge, **bs** – base, **dpr** – distal process, **lgr** – lateral grooves, **lpr** – lateral process, **mbr** – membrane, **mel** – median elevation, **mp** – median process, **ota** – oval-shaped translucent area, **sh** – shoulder, **spi** – spiculum, **wg** – wing.

K. Zenz 2018“ [printed], “Lectotype Colletes succinctus brevigena Noskiewicz, 1936 des. Katharina Zenz et al. 2020” [printed on red paper].

Paralectotypes deposited in the Natural History Museum Vienna: 1 ♂ (legs partly broken) labelled as the lectotype; 1 ♂ (legs and antennae partly broken) labelled “Pola Schlett.” [= Pula, today in Croatia, leg. Schletterer; printed]; 8 ♂♂ (in various conditions) labelled “Transkauk. Helenendorf 1886.” [= Goygol in Azerbaijan; printed “6” on some labels handwritten]; all specimens with Noskiewicz’s and Zenz’s identification label as the lectotype and a type label “Paralectotype Colletes succinctus brevigena Noskiewicz, 1936 labelled by K. Zenz 2020” [printed on red paper].

Specific characters of terminal structures and genitalia of the male *C. brevigena*:

Sternum 7: Very pronounced shoulders (**sh**) with broad basal arms (**ba**), densely hairy. Concavely curved distal margin of membrane (**mbr**) bearing few hairs (Fig. 13B). – Sternum 8: Spiculum (**sp**) with a broad, sclerotised bifurcation (Fig. 13C, D). – Sternum 6 and genital capsule: no species-specific characters were detected (Figs 13A, 14).

For species-specific characters of the male terminal structures and genitalia of *C. pannonicus*, see the next chapter.

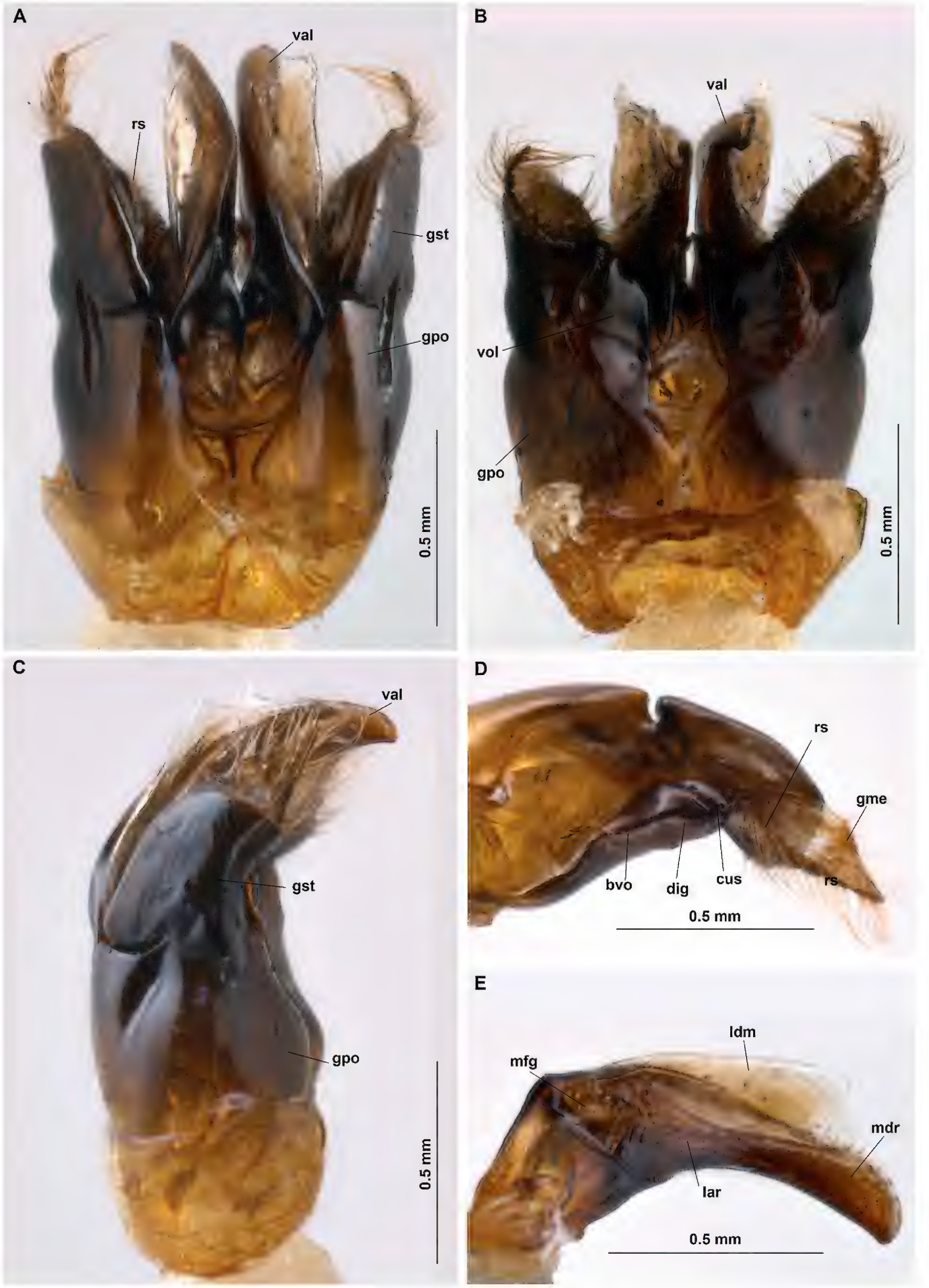

Figure 7. Male genitalia of *Colletes succinctus*, specimen no. 243 (LM): **A.** Dorsal view of genital capsule; **B.** Ventral view of genital capsule; **C.** Lateral view of genital capsule; **D.** Mediolateral view of gonopod and gonostylus with volsella; **E.** Lateral view of penis valva. **blg** – basolateral groove, **bvo** – basivolsella, **cus** – cuspis, **dig** – digitus, **gme** – gonostylus membrane, **gpo** – gonopod, **gst** – gonostylus, **lar** – lateral area, **ldm** – laterodorsal membrane, **mdr** – mesodorsal ridge, **rs** – row of setae, **val** – penis valva, **vol** – volsella.

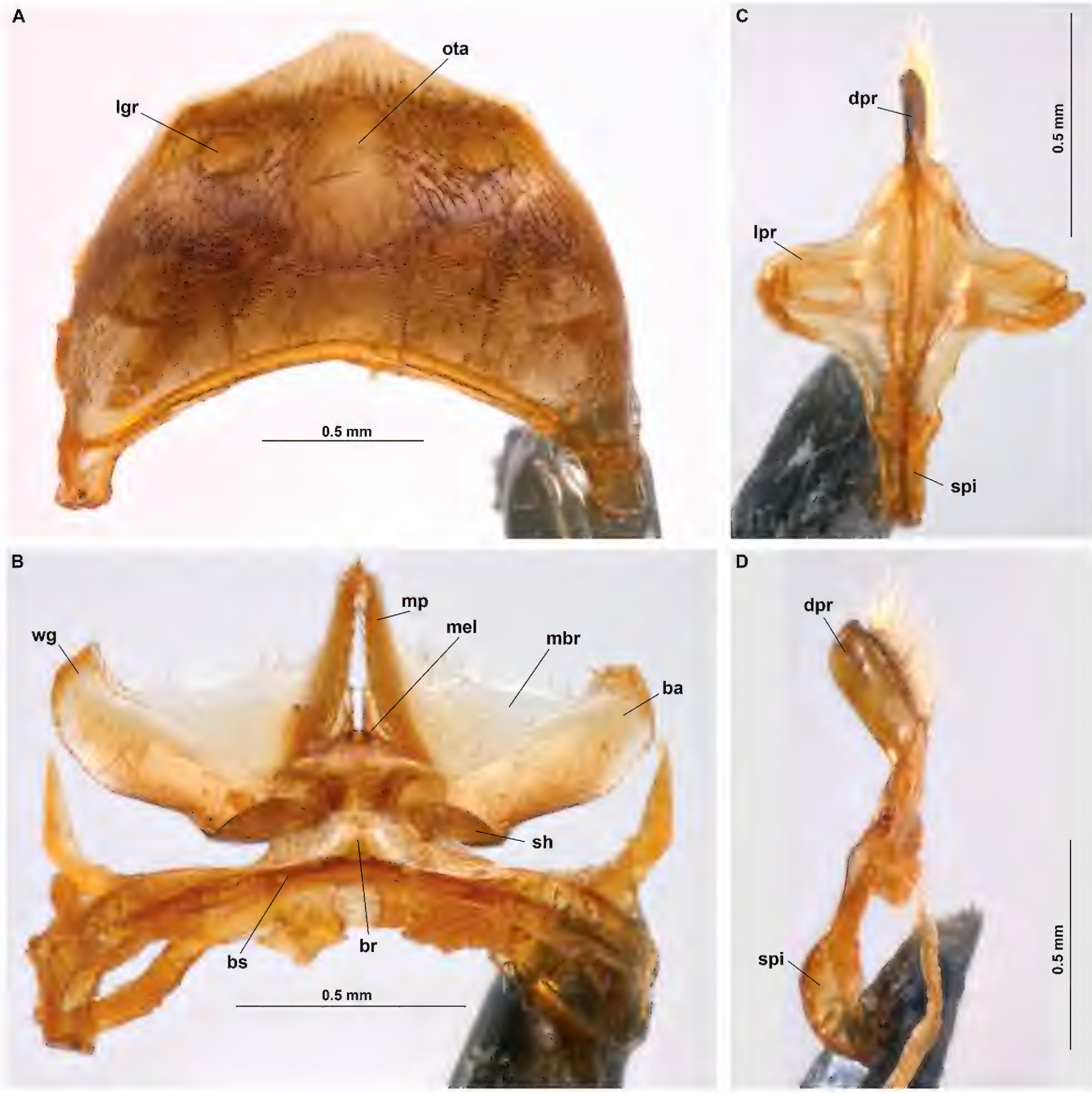

Figure 8. Male terminal structures of *Colletes halophilus*, specimen no. 180 (LM): **A.** Ventral view of sternum 6; **B.** Ventral view of sternum 7; **C.** Ventral view of sternum 8; **D.** Lateral view of sternum 8. **ba** – basal arm, **br** – bridge, **bs** – base, **dpr** – distal process, **lgr** – lateral grooves, **lpr** – lateral process, **mbr** – membrane, **mel** – median elevation, **mp** – median process, **ota** – oval-shaped translucent area, **sh** – shoulder, **spi** – spiculum, **wg** – wing.

First description of the male of *C. pannonicus*

Colletes pannonicus is a recently described species that has been solely found in the Seewinkel near Lake Neusiedl (Hölzler and Mazzucco 2011). The "preliminary description" was based on a female specimen (holotype) and since the males of *C. pannonicus* have not yet been described, this is done on the following, based on dry specimens collected sympatrically with *C. pannonicus* females in Seewinkel on *Tripolium pannonicum* (sea aster).

Examined material. Austria, Burgenland: 1 ♂, Podersdorf, 4.9.1991, leg. M. Madl, coll. H. Zettel (spec. no. 174); 1 ♂, Illmitz, Hölle; 4.9.2006, leg. & coll. H. Wiesbauer (spec.no. 166), 1 ♂, Podersdorf, 9.9.2012, leg. & coll. H. Wiesbauer (spec.no. 175); 1 ♂, Podersdorf; 29. 8.2014, leg. & coll. H. Wiesbauer (spec.no. 176); 2 ♂♂, Illmitz, Hölle, 29–30.8.2014, leg. & coll. H. Wiesbauer (spec.no. 167–168); 1 ♂, Neusiedl am See, Kalvarienberg, 47°56'33.69"N, 16°51'39.23"E, 8. 9.2016, leg. & coll. L.W. Gunczy (spec.no. 171).

Description. Face with long yellow-whitish hair. Galea reticulated between sensilla, segments of maxillary palpi long and narrow. Mesonotum coarsely and densely punctured, in some specimens with a shiny centre of varying size, with long orange-brownish hair. Mesopleura densely punctured, (sporadically) punctures

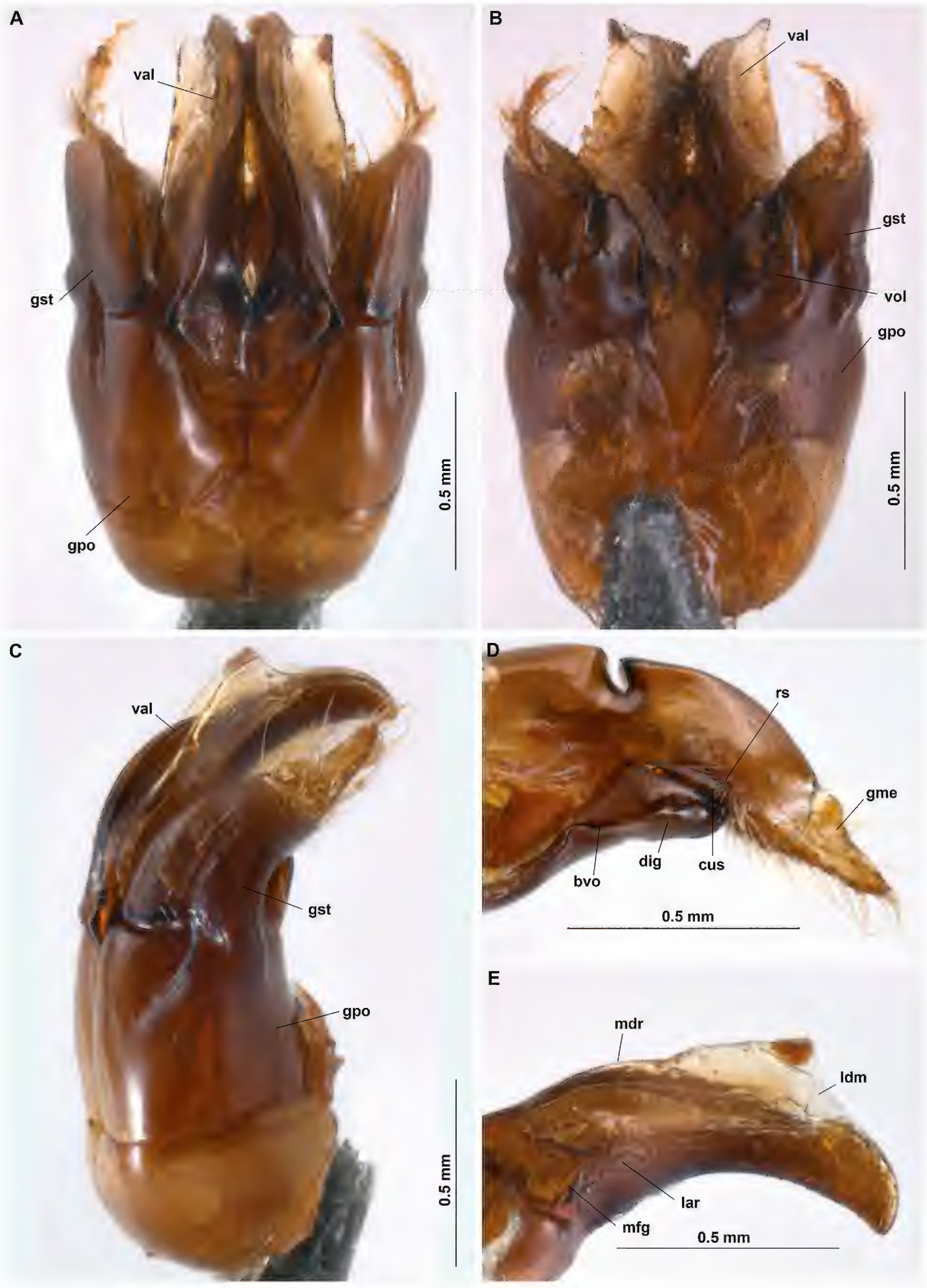

Figure 9. Male genitalia of *Colletes halophilus*, specimen no. 180 (LM): **A.** Dorsal view of genital capsule; **B.** Ventral view of genital capsule; **C.** Lateral view of genital capsule; **D.** Mediolateral view of gonopod and gonostylus with volsella; **E.** Lateral view of penis valva. **blg** – basolateral groove, **bvo** – basivolsella, **cus** – cuspis, **dig** – digitus, **gme** – gonostylus membrane, **gpo** – gonopod, **gst** – gonostylus, **lar** – lateral area, **ldm** – laterodorsal membrane, **mdr** – mesodorsal ridge, **rs** – row of setae, **val** – penis valva, **vol** – volsella.

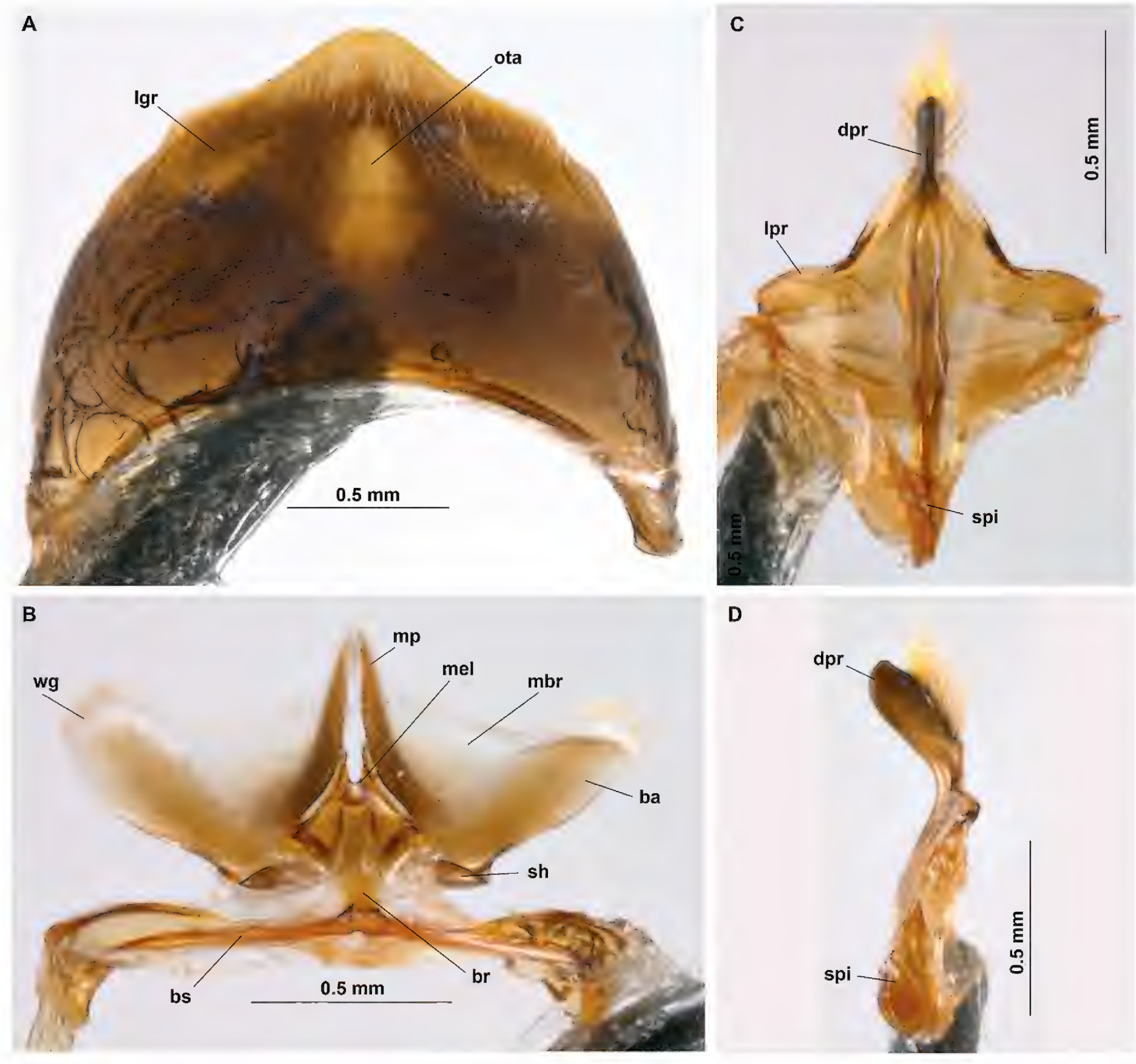

Figure 10. Male terminal structures of *Colletes hederae*, specimen no. 197 (LM): **A.** Ventral view of sternum 6; **B.** Ventral view of sternum 7; **C.** Ventral view of sternum 8; **D.** Lateral view of sternum 8. **ba** – basal arm, **br** – bridge, **bs** – base, **dpr** – distal process, **lgr** – lateral grooves, **lpr** – lateral process, **mbr** – membrane, **mel** – median elevation, **mp** – median process, **ota** – oval-shaped translucent area, **sh** – shoulder, **spi** – spiculum, **wg** – wing.

merge and form wrinkles, with yellow-whitish hair. Hair on propodeum yellowish-white to yellow-orange coloured. Stripes of setae on terga yellow-whitish. Punctures on tergum 1 large, but smaller than on mesonotum. Tergum 1 deeply and coarsely punctured, on disc dense, to the sides with interspaces of the size of 0.5–1 puncture diameter; cuticle shiny; basal declivity with long yellow hair. Tergum 2 densely punctured, sporadically with intervals of the size of one puncture diameter; cuticle shiny (Fig. 15D).

Specific characters of terminal structures and genitalia of the male *C. pannonicus*:

Sternum 6: Lateral grooves (**lgr**) oval and large (Fig. 16A). – Sternum 7: Slender wings (**wg**), with setae on their proximal half. Hairless membrane (**mbr**), sigmoid-curved hind margin bearing short hairs (Fig. 16B). – Sternum 8: Distal process (**dpr**) basal with outwardly curved shoulders, with sclerotised, short and discontinuous median bifurcation (Fig. 16C). – Genital capsule: no species-specific characters were detected (Fig. 17).

Identification keys

Using a range of external morphological characters (Table 1), as well as the identified differences in male genitalia, it was possible to establish identification keys for both females and males. All investigated specimens were subjected to a detailed examination and determined according to the following identification keys for females and males:

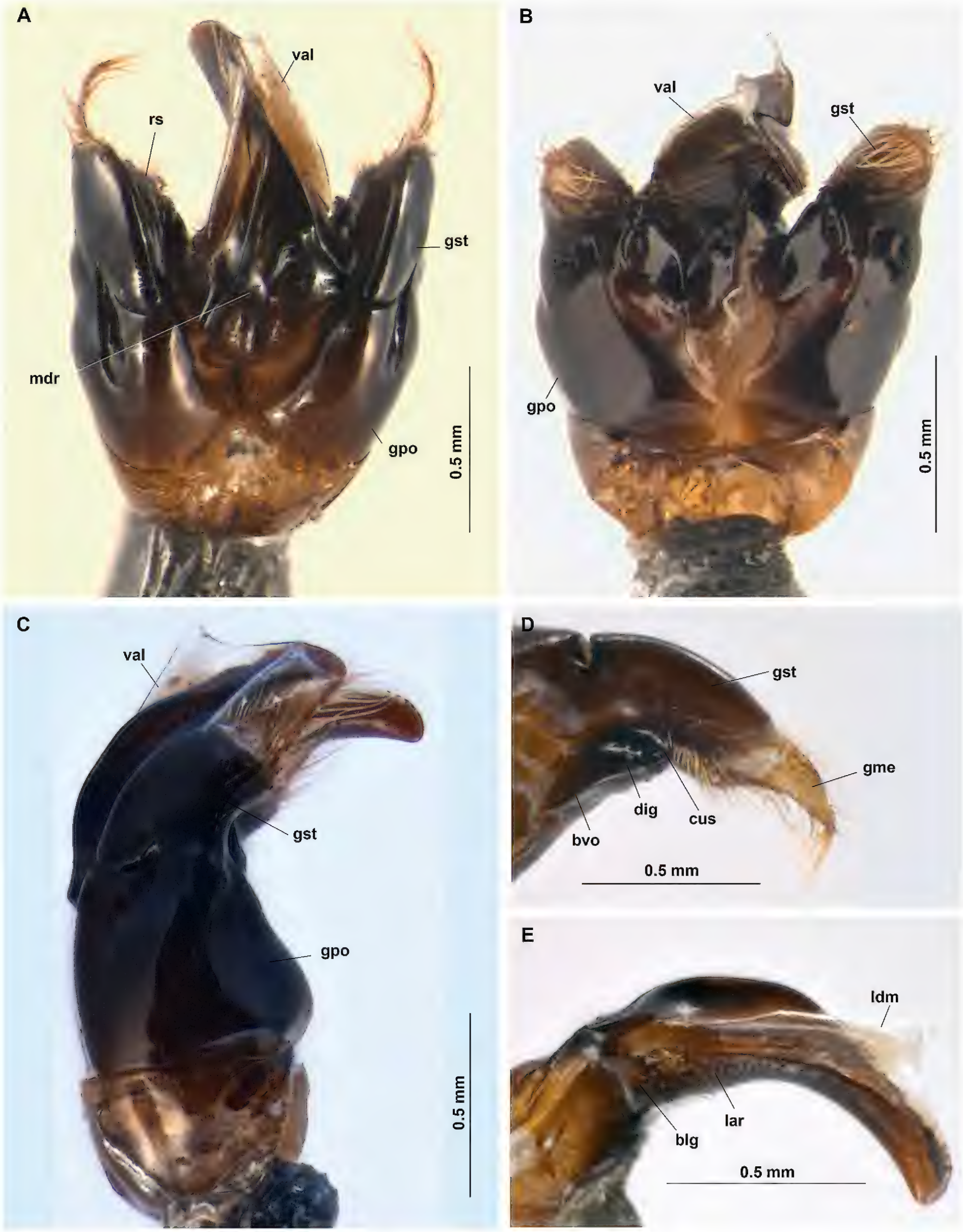

Figure 11. Male genitalia of *Colletes hederae*, specimen no. 197 (LM): **A.** Dorsal view of genital capsule; **B.** Ventral view of genital capsule; **C.** Lateral view of genital capsule; **D.** Mediolateral view of gonopod and gonostylus with volsella; **E.** Lateral view of penis valva. **blg** – basolateral groove, **bvo** – basivolsella, **cus** – cuspis, **dig** – digitus, **gme** – gonostylus membrane, **gpo** – gonopod, **gst** – gonostylus, **lar** – lateral area, **ldm** – laterodorsal membrane, **mdr** – mesodorsal ridge, **rs** – row of setae, **val** – penis valva, **vol** – volsella.

Figure 12. Male of *Colletes brevigena*, lectotype: **A.** Lateral view; **B.** Dorsal view; **C.** Frontal view; **D.** Terga 1 and 2. **T1** – tergum 1, **T2** – tergum 2.

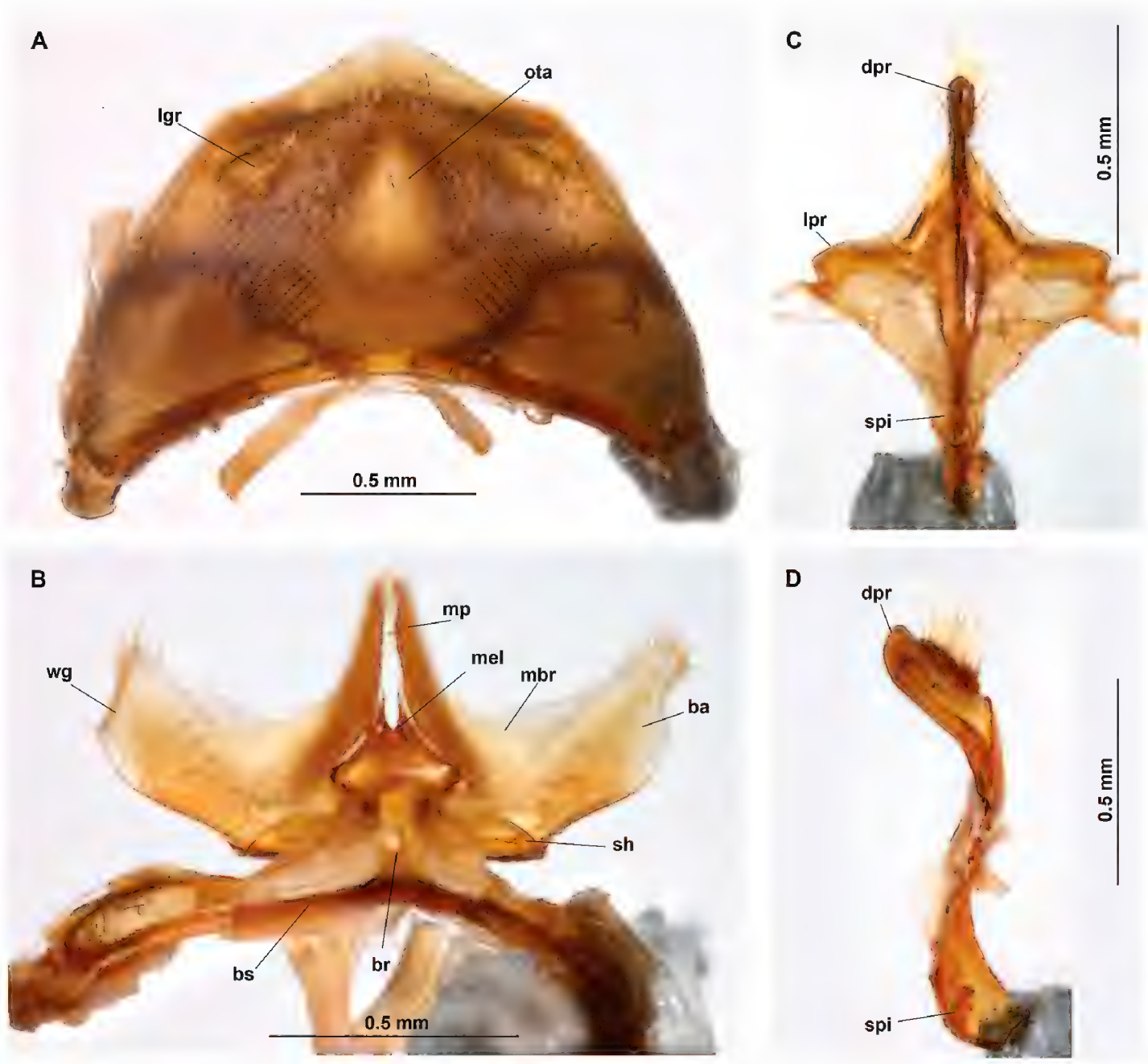

Figure 13. Male terminal structures of *Colletes brevigena*, specimen no. 270 (LM): **A.** Ventral view of sternum 6; **B.** Ventral view of sternum 7; **C.** Ventral view of sternum 8; **D.** Lateral view of sternum 8. **ba** – basal arm, **br** – bridge, **bs** – base, **dpr** – distal process, **lgr** – lateral grooves, **lpr** – lateral process, **mbr** – membrane, **mel** – median elevation, **mp** – median process, **ota** – oval-shaped translucent area, **sh** – shoulder, **spi** – spiculum, **wg** – wing.

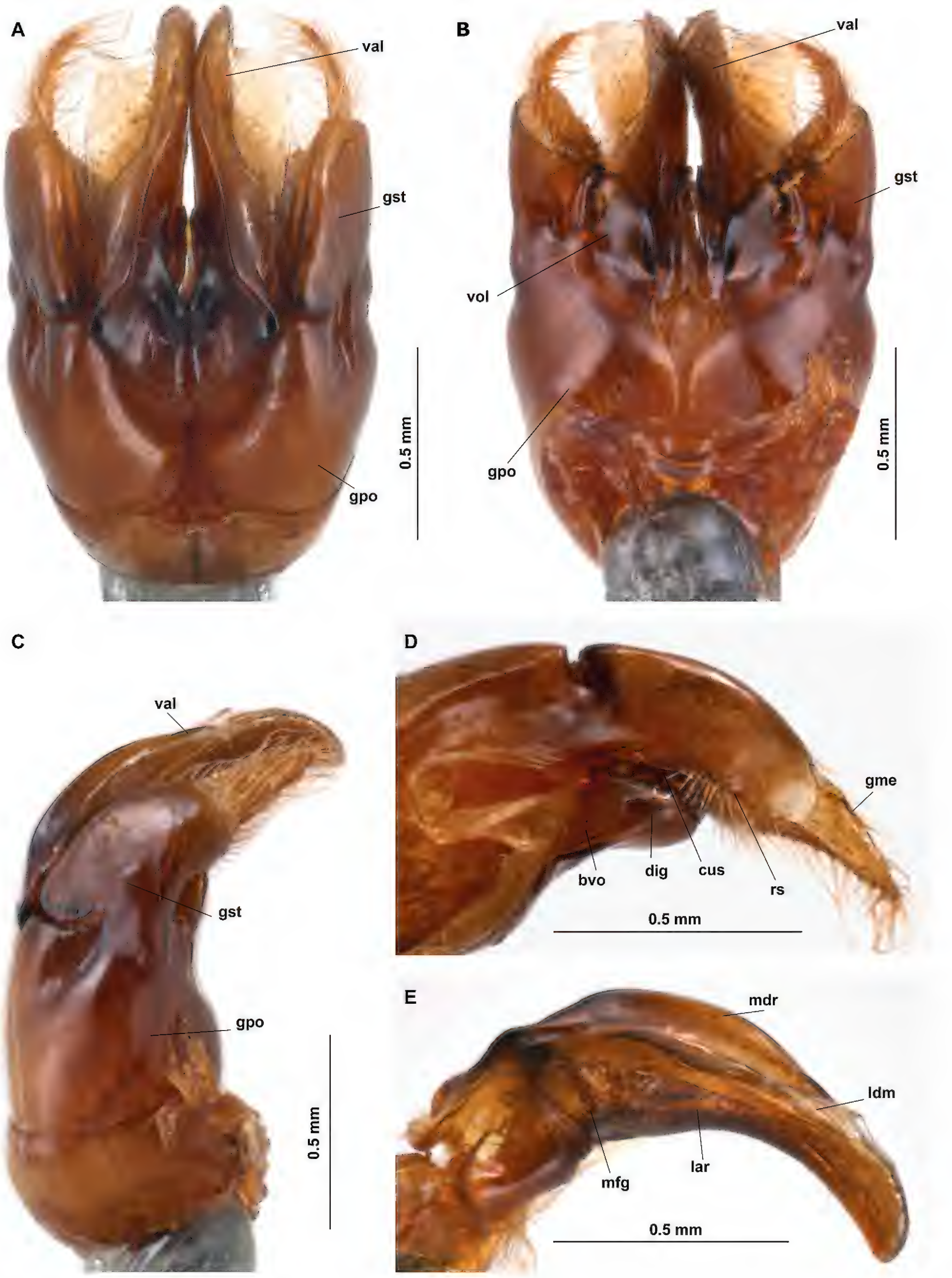

Figure 14. Male genitalia of *Colletes brevigena*, specimen no. 270 (LM): **A.** Dorsal view of genital capsule; **B.** Ventral view of genital capsule; **C.** Lateral view of genital capsule; **D.** Mediolateral view of gonopod and gonostylus with volsella; **E.** Lateral view of penis valva. **lg** – basolateral groove, **bvo** – basivolsella, **cus** – cuspis, **dig** – digitus, **gme** – gonostylus membrane, **gpo** – gonopod, **gst** – gonostylus, **lar** – lateral area, **ldm** – laterodorsal membrane, **mdr** – mesodorsal ridge, **rs** – row of setae, **val** – penis valva, **vol** – volsella.

Figure 15. Male of *Colletes pannonicus*: **A.** Lateral view; **B.** Dorsal view; **C.** Frontal view; **D.** Terga 1 and 2. **T1** – tergum 1, **T2** – tergum 2.

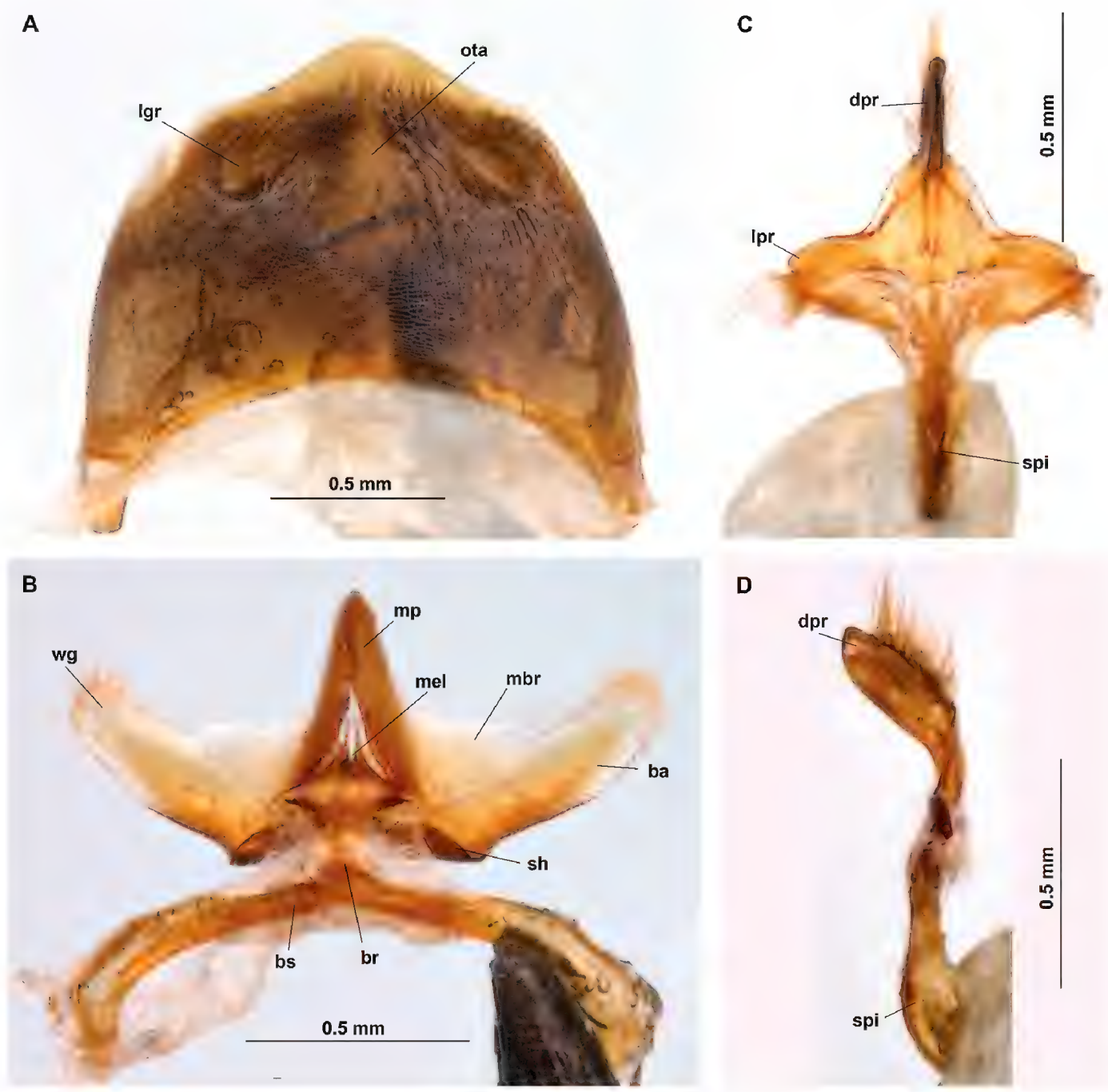

Figure 16. Male terminal structures of *Colletes pannonicus*, specimen no. 171 (LM): **A.** Ventral view of sternum 6; **B.** Ventral view of sternum 7; **C.** Ventral view of sternum 8; **D.** Lateral view of sternum 8. **ba** – basal arm, **br** – bridge, **bs** – base, **dpr** – distal process, **lgr** – lateral grooves, **lpr** – lateral process, **mbr** – membrane, **mel** – median elevation, **mp** – median process, **ota** – oval-shaped translucent area, **sh** – shoulder, **spi** – spiculum, **wg** – wing.

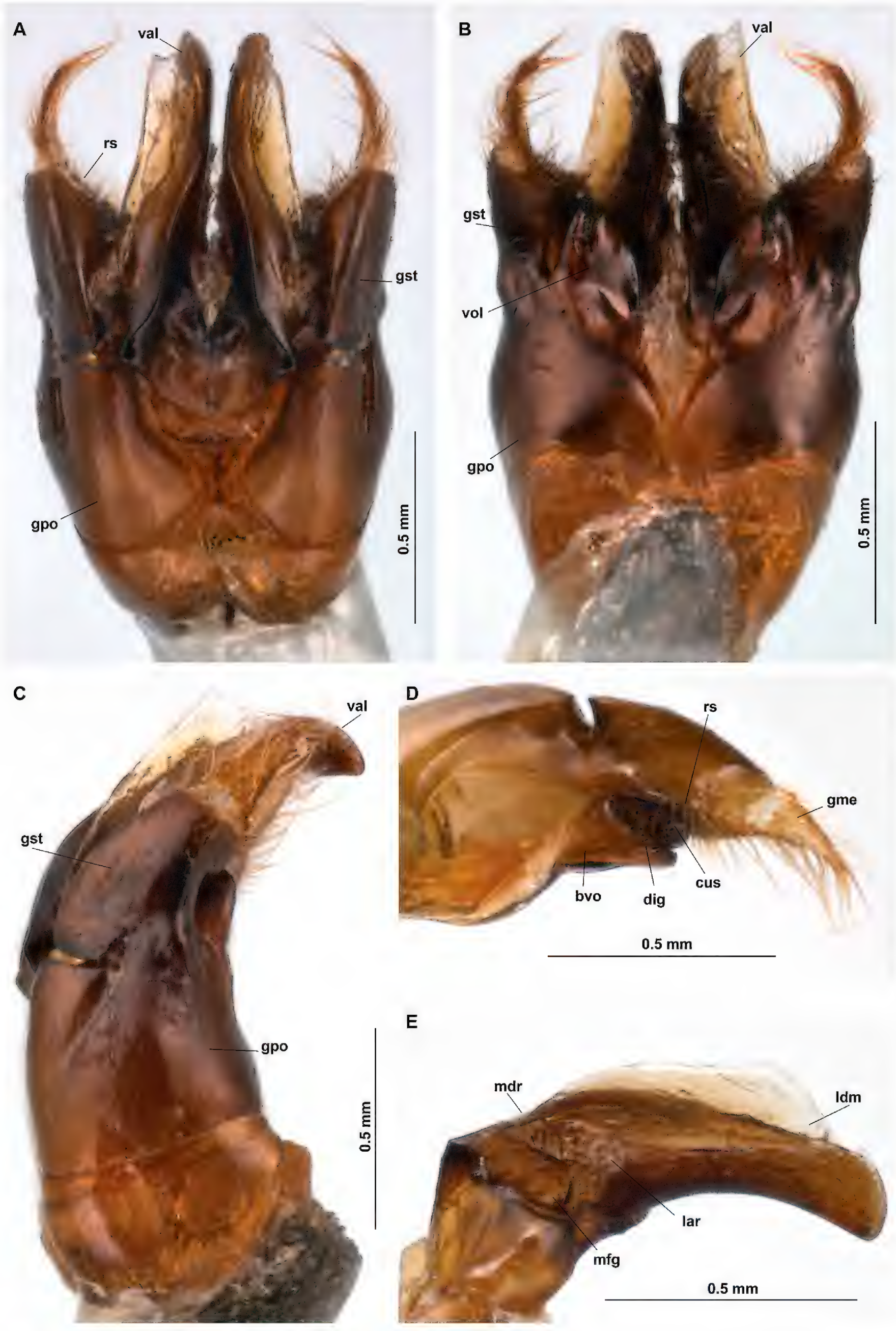

Figure 17. Male genitalia of *Colletes pannonicus*, specimen no. 171 (LM): **A.** Dorsal view of genital capsule; **B.** Ventral view of genital capsule; **C.** Lateral view of genital capsule; **D.** Mediolateral view of gonopod and gonostylus with volsella; **E.** Lateral view of penis valva. **blg** – basolateral groove, **bvo** – basivolsella, **cus** – cuspis, **dig** – digitus, **gme** – gonostylus membrane, **gpo** – gonopod, **gst** – gonostylus, **lar** – lateral area, **ldm** – laterodorsal membrane, **mdr** – mesodorsal ridge, **rs** – row of setae, **val** – penis valva, **vol** – volsella.

Identification key to females of the Austrian species of the *C. succinctus* group

1 Apical tergal hair bands narrow over entire width and more narrowed towards the middle (Fig. 3E). Propodeum hairless in centre of declivity. Mesonotum continuously punctured with scattered black-brown hair in the middle. Puncture at mesopleura separated by intervals that are two or three times larger than diameter of punctures. Cuticle of frons reticulated and punctured (Fig. 3F). Clypeus with longitudinal wrinkles that (sporadically) incline inwards towards the end (Fig. 18A). Galea with microstructure between sensilla (Fig. 14C, D). Narrow, elongated fovea facialis with slightly pointed apex (Figs 3D, 18B) ..... *C. collaris*

– Stripes of hair at the posterior margins of gaster terga wide..... 2

2 Clypeus with mesally curved longitudinal wrinkles, the most lateral 1–2 wrinkles of each side meeting each other in the middle behind fore-margin (Fig. 19A). Tergum 1 densely and finely punctured, punctures becoming abruptly smaller towards hind margin. Tergum 2 with slit-shaped structure, slits fine and loosely standing next to each other (Fig. 3K). Mesonotum densely punctured with shiny centre, variable in its size. Mesopleura densely punctured, punctures can merge and form wrinkles. Galea with microstructure between sensilla (Fig. 19C). Upper end of fovea facialis rounded, slightly kidney-shaped (Figs 3J, 19B)..... *C. hederae*

– Wrinkles on the clypeus straight, longitudinal or slightly inclined mesally near apex, never meeting each other in middle ...3

3 Galea between sensilla shiny, without microreticulation. Segments of the maxillary palpi short and stout (Figs 3C, 20C, D). Mesonotum densely punctured with shiny area in its centre. Mesopleura densely punctured with scattered intervals of the diameter of puncture. Tergum 1 sparsely punctured and slowly shrinking towards the distal end, distance of puncture on disc as long as diameter of punctures. Tergum 2 coarse and densely, oval punctured (Fig. 3B). Clypeus with longitudinal wrinkles, at most slightly inclined mesally at distal end; with transverse, long furrow at anterior margin (Fig. 20A). Fovea facialis elongated, narrow, with oval-shaped margin at the upper end (Figs 3A, 20A) ..... *C. succinctus*

– Galea with microreticulation between sensilla. Segments of maxillary palpi strongly elongated 4

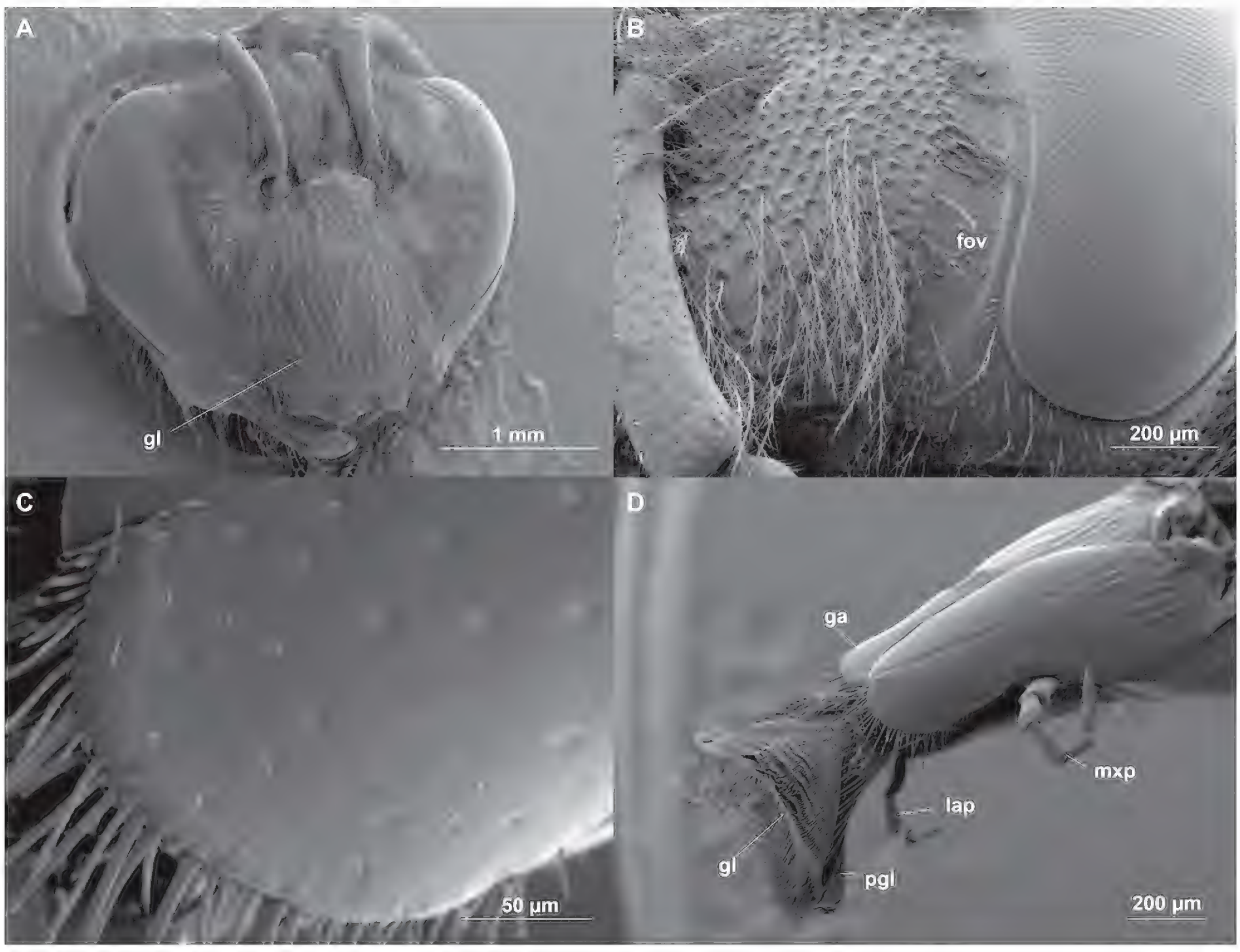

Figure 18. Head of a female of *Colletes collaris*, no. 190 (SEM): **A.** Latero-frontal view of head; **B.** Upper part of fovea facialis in dorsal view, right antenna on the left; **C.** Detailed view of setae and microstructure of galea; **D.** Latero-dorsal view of proboscis. **ant** – antenna, **ce** – complex eye, **cl** – clypeus, **fov** – fovea facialis, **ga** – galea, **gl** – glossa, **lap** – labial palp, **mxp** – maxillary palp, **pgl** – paraglossa, **scl** – supra-clypeus, **sen** – sensilla.

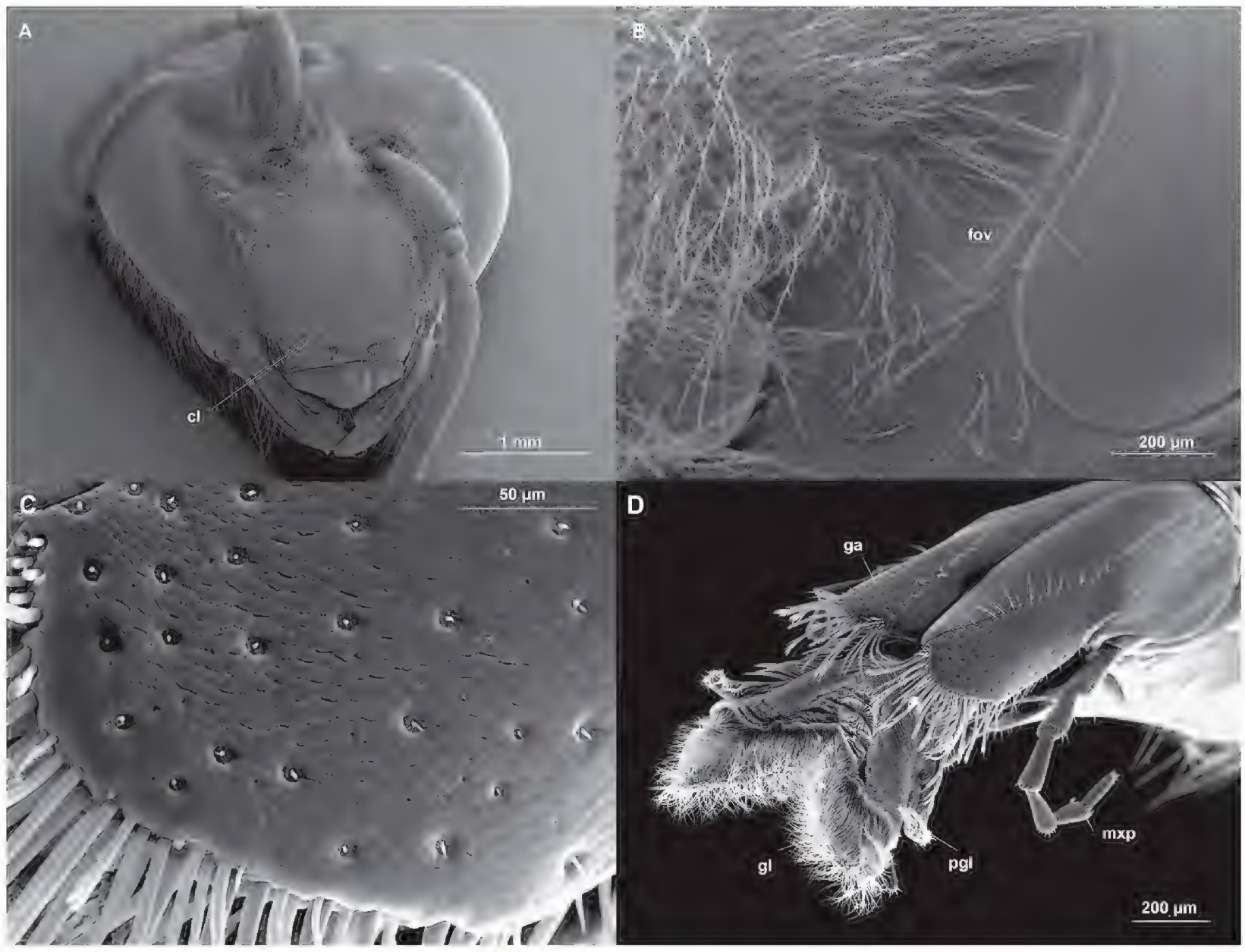

Figure 19. Head of a female of *Colletes hederae*, no. 202 (SEM): **A.** Latero-frontal view of head; **B.** Upper part of fovea facialis in dorsal view, lateral ocellus on the left; **C.** Detailed view of setae and microstructure of galea; **D.** Latero-dorsal view of proboscis. **ce** – complex eye, **cl** – clypeus, **fov** – fovea facialis, **ga** – galea, **gl** – glossa, **mxp** – maxillary palp, **oc** – lateral ocellus, **pgl** – paraglossa, **scl** – supra-clypeus, **sen** – sensilla.

4 Fovea facialis widening towards upper end, with deep, broad and straight margin (Figs. 3M and 21B). Mesonotum densely punctured, distance of punctures on disc up to twice as long as diameter of punctures, the sparsely punctured area can vary in its size. Mesopleura densely punctured. Tergum 1 with dense, large punctures, (sporadically) distance of puncture as long as diameter of punctures. Tergum 2 very densely punctured (Fig. 3N). Clypeus with longitudinal wrinkles, distal wrinkles slightly inclined to the middle (Figs. 3O and 21A) ..... *C. pannonicus*

– Fovea facialis widening towards the upper end and tapering to a medio-lateral point, outer margin more depressed than inner margin (Figs 3G, 22B). Mesonotum and mesopleura densely punctured (Fig. 3I). Tergum 1 densely punctured, (sporadically) with distances of punctures as long as diameter of punctures. Tergum 2 punctured (Fig. 3H). Clypeus longitudinally wrinkled (Figs 22A, D) ..... *C. brevigena*

Identification key to males of the Central European species of the *C. succinctus* group

1 Narrow stripes of hair at the posterior margin of the terga. Propodeum hairless in centre of declivity. Distal processes of sternum 7 short (wg), transverse-oval, medially not extended, but broadly separated from each other (mel), completely densely haired (Fig. 4B). Mesonotum with dense puncturation. Mesopleura sporadically punctured, with intervals twice the size of a diameter. Galea with microstructure between sensilla. Stripes of hair on terga narrowed towards the middle. Tergum 1 densely punctured, partly with diameter-sized intervals in-between punctures. Tergum 2 with coarse puncturation, intervals about the size of 1–2 diameters. Tergum 3 coarsely punctured with over diameter-sized intervals ..... *C. collaris*

– Broad stripes of hair at the posterior margin of the terga 2

2 Galea between sensilla shiny without microreticulation. Segments of the maxillary palpi short and stout. Hind margins of membrane (mbr) of distal processes of sternum 7 (wg) almost straight and with long setae. Wings (wg) very broad (Fig. 6B). Mesonotum coarsely punctured, with smooth area in its centre. Mesopleura coarsely and densely punctured, with diameter-sized distances of punctures. Tergum 1 densely punctured, with intervals of a puncture diameter. Puncturation of tergum 2 roundish, smaller than on tergum 1. Tergum 3 puncturation with intervals of double or triple diameters ..... *C. succinctus*

– Cuticle of galea between sensilla reticulated. Segments of the maxillary palpi long and slender. Hind margins of the wing-shaped processes of sternum 7 (wg) concave and with short setae. Wings (wg) narrow 3

3 In lateral view membrane of gonostylus (gme) of genital arched dorsally, with dorsobasal knob and densely haired (Fig. 9D). Mesonotum coarsely punctured, with smooth area in its centre, varying in its size. Mesopleura very densely punctured, punctures merge into each other, wrinkled. Galea with reticulate structure between sensilla. Tergum 1 densely punctured, intervals about the size of a diameter. Puncturation of tergum 2 round and dense, of tergum 3 slit-shaped and dense ..... *C. halophilus*

– In lateral view, gonostylus base of genital straight and hairy. 4

4 Base of basal arms (ba) of wings only half as wide as apex. Membrane of wings (mbr) concave, proximally densely hairy (Fig. 10B). Mesonotum densely punctured, sometimes with a smooth area in its centre. Mesopleura densely punctured, slightly wrinkled. Galea with reticulate structure between sensilla. Tergum 1 densely and finely punctured. Puncturation on tergum 2 dense and oval-shaped, finer than on tergum 1, of tergum 3 densely and slit-like structured..... *C. hederae*

– Base of basal arms (ba) of wings almost as broad as apex. 5

5 Basal arms (ba) completely densely hairy, membrane (mbr) sparsely hairy (Fig. 13B). Mesonotum coarsely punctured, sometimes with a smooth area of varying size in its middle. Mesopleura very densely punctured. Galea with reticulate structure between sensilla. Tergum 1 with coarse and large punctures. Puncturation of tergum 2 dense and roundish, smaller than on tergum 1 ..... *C. brevigena*

– Basal arms (ba) haired in lower half, membrane (mbr) hairless (Fig. 16B). Puncturation on mesonotum dense and coarse, sometimes with shiny centre. Mesopleura densely punctured, sometimes with wrinkles. Galea with reticulate microstructure between sensilla. Tergum 1 coarse and large punctured. Tergum 2 coarse and densely punctured, round and smaller than on tergum 1 ..... *C. pannonicus*

Non-assignable specimens

All studied specimens were subjected to a control of their species affiliation. The specimens were re-identified with the help of the new key presented herein. A total of 21 (19 females and two males) of the 270 specimens were assigned to another species of the *C. succinctus* group than previously. Three males were excluded from the *Colletes succinctus*

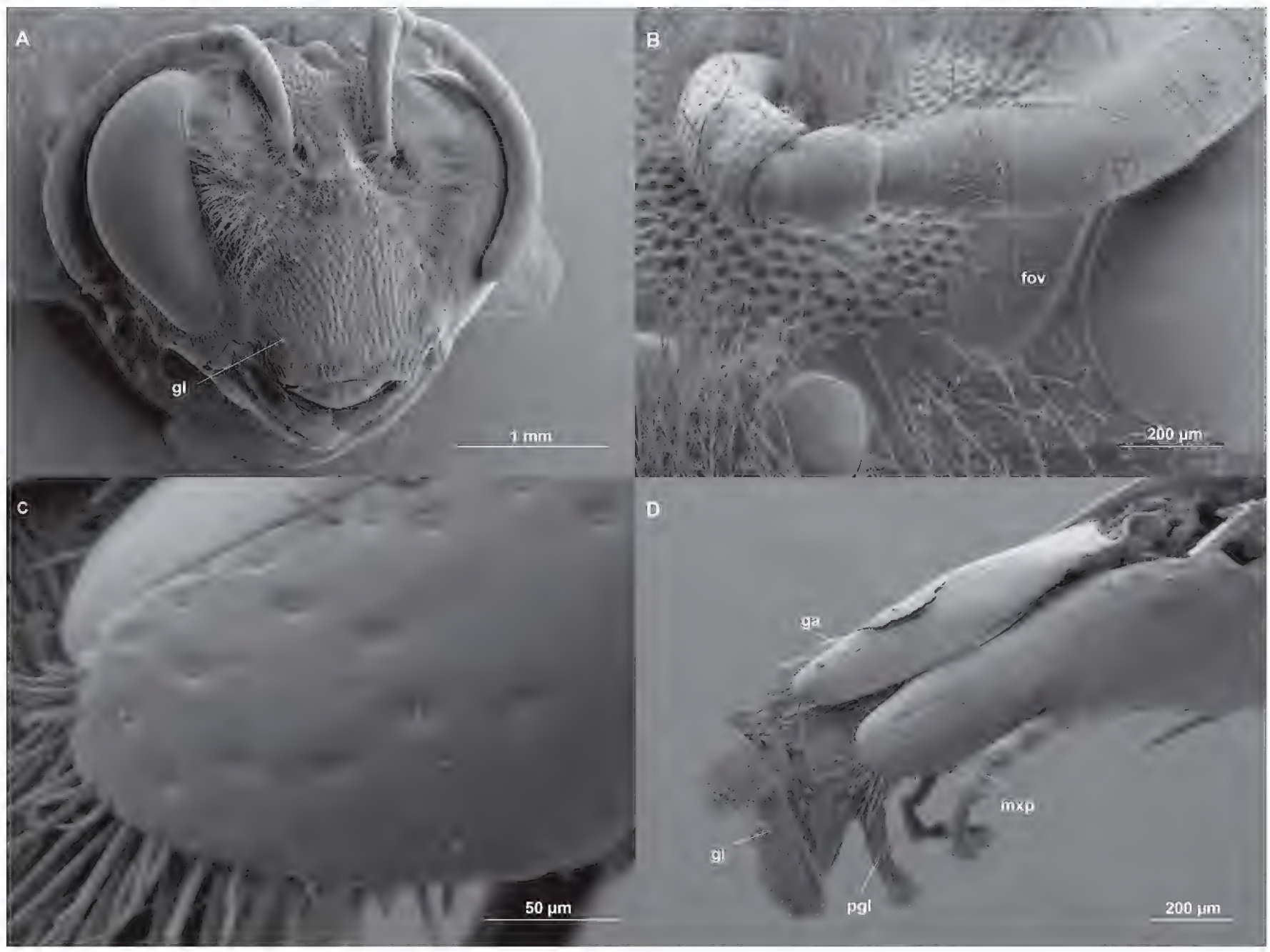

Figure 20. Head of a female of *Colletes succinctus*, no. 237 (SEM): **A.** Latero-frontal view of head; **B.** Upper part of fovea facialis in dorsal view, lateral ocellus on the left; **C.** Detailed view of setae and microstructure of galea; **D.** Latero-dorsal view of proboscis. **ant** – antenna, **ce** – complex eye, **cl** – clypeus, **fov** – fovea facialis, **ga** – galea, **gl** – glossa, **mxp** – maxillary palp, **oc** – ocellus, **pgl** – paraglossa, **sen** – sensilla, **scl** – supra-clypeus.

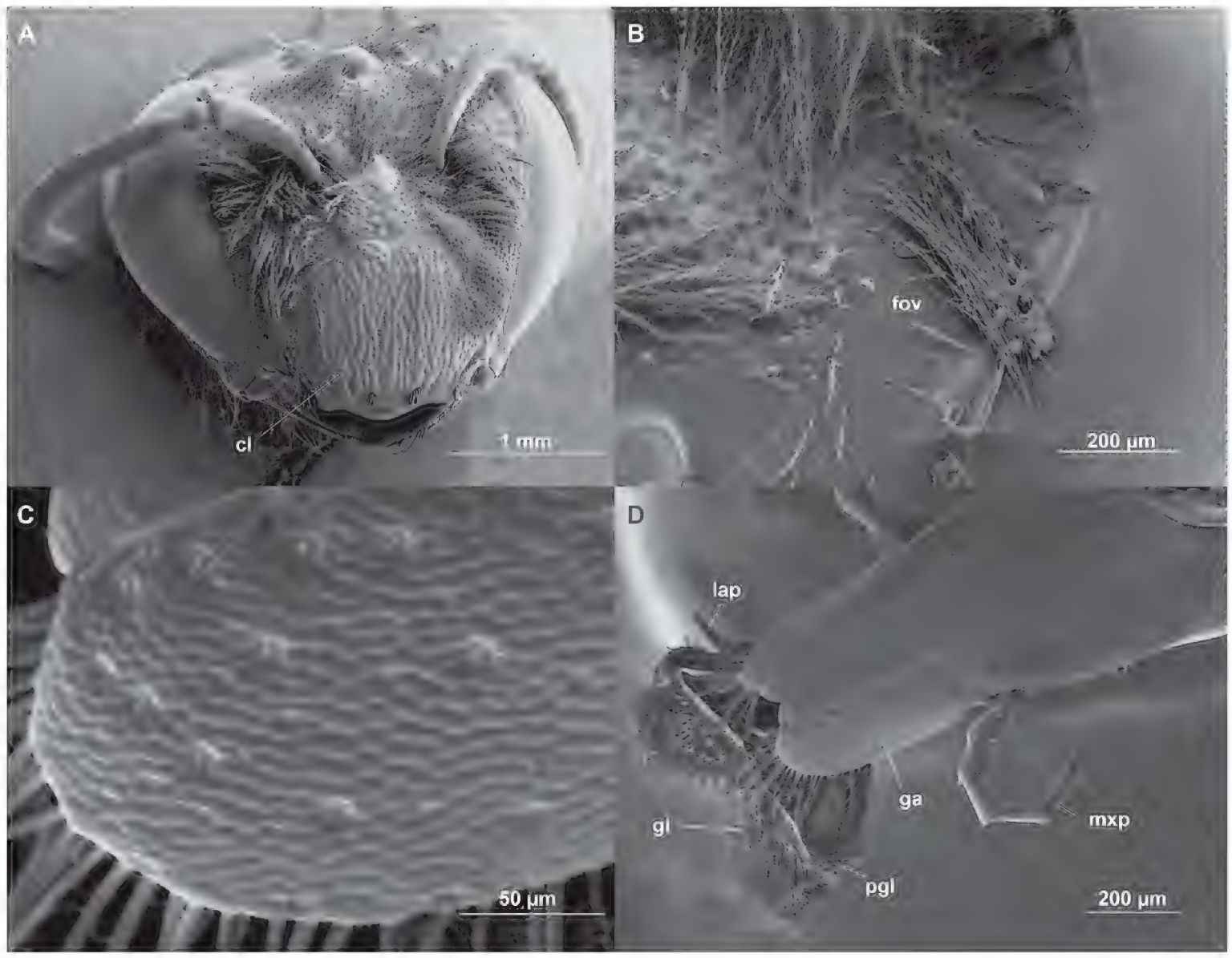

Figure 21. Head of a female of *Colletes pannonicus*, no. 232 (SEM): **A.** Latero-frontal view of head; **B.** Upper part of fovea facialis in dorsal view, lateral ocellus on the left; **C.** Detailed view of setae and microstructure of galea; **D.** Latero-dorsal view of proboscis. **cl** – clypeus, **ce** – complex eye, **fov** – fovea facialis, **ga** – galea, **gl** – glossa, **lap** – labial palp, **mxp** – maxillary palp, **oc** – lateral ocellus, **pgl** – paraglossa, **sen** – sensilla, **scl** – supra-clypeus.

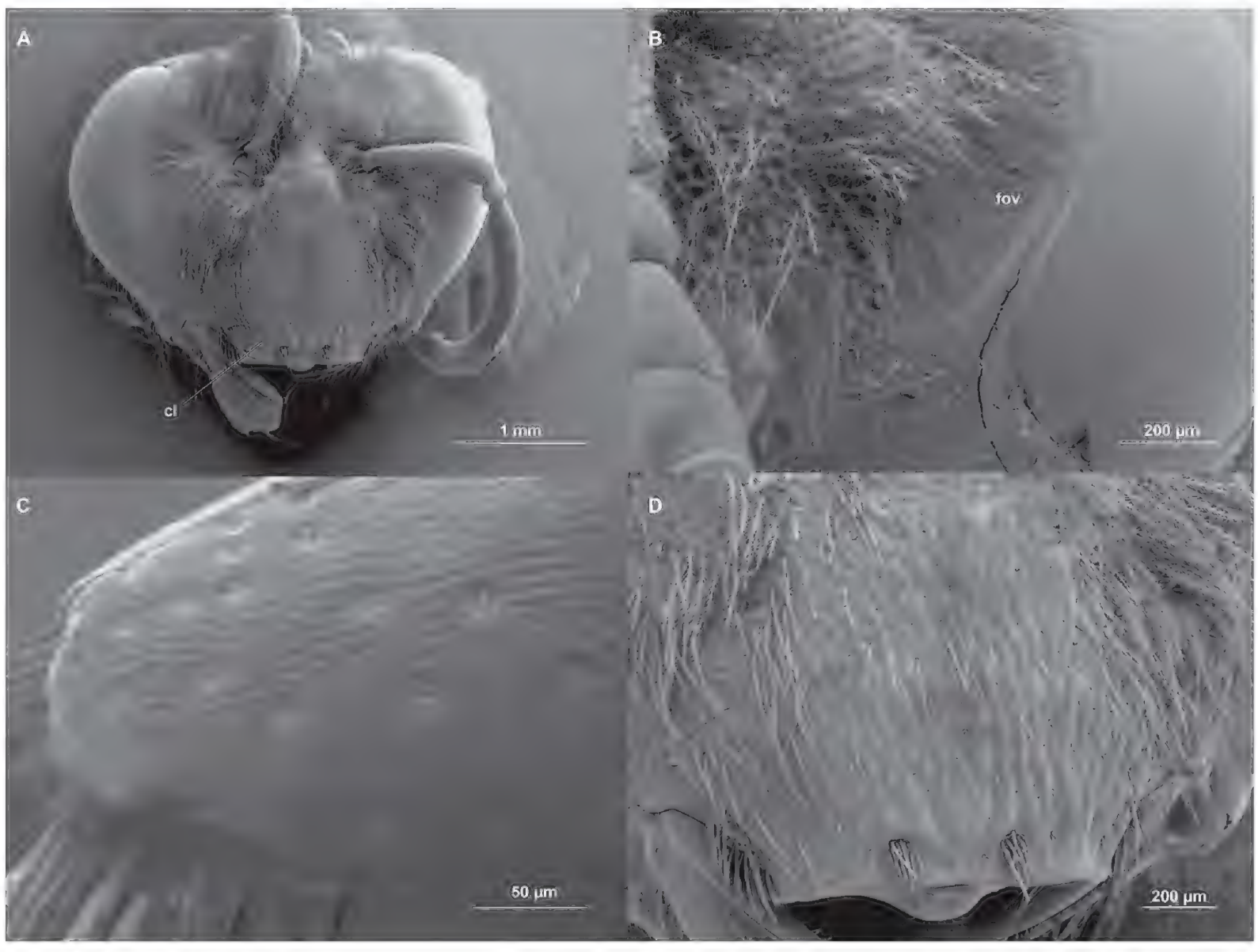

Figure 22. Head of a female of *Colletes brevigena*, no. 190 (SEM): **A.** Latero-frontal view of head; **B.** Upper part of fovea facialis in dorsal view, lateral ocellus and antenna on the left; **C.** Detailed view of setae and microstructure of galea; **D.** Latero-dorsal view of proboscis. **ant** – antenna, **ce** – complex eye, **cl** – clypeus, **fov** – fovea facialis, **oc** – lateral ocellus, **sen** – sensilla, **scl** – supra-clypeus.

Table 3. Mean, maximum and minimum values of all measured distances (in millimetres) for six European species of the *Colletes succinctus* group (females; n = 103). Measured distances: (HL) head length, (HW) head width, (EL) eye length, (CL) clypeus length, (UID) upper interocular distance, (LID) lower interocular distance, (MID) middle interocular distance, (CHL) cheek length, (TW) thorax width.

	C. succinctus (n = 26)	*C. collaris* (n = 20)	*C. brevigena* (n = 14)	*C. halophilus* (n = 10)	*C. hederae* (n = 22)	*C. pannonicus* (n = 11)
Mean (HL)	2.60	2.69	2.73	2.76	2.90	2.60
Min. (HL)	2.35	2.47	2.51	2.59	2.59	2.47
Max. (HL)	2.76	3.00	2.92	3.05	3.09	2.76
Mean (HW)	3.39	3.42	3.60	3.64	3.72	3.5
Min. (HW)	3.06	3.19	3.28	3.44	3.47	3.28
Max. (HW)	3.70	3.83	3.9	3.83	3.99	3.64
Mean (EL)	2.00	2.00	2.17	2.13	2.19	2.05
Min. (EL)	1.88	1.85	1.98	1.98	2.05	1.92
Max. (EL)	2.24	2.24	2.31	2.27	2.31	2.14
Mean (CL)	1.07	1.10	1.13	1.17	1.23	1.10
Min. (CL)	0.83	0.95	1.03	1.06	1.13	1.03
Max. (CL)	1.21	1.24	1.31	1.31	1.34	1.16
Mean (UID)	1.99	2.03	2.12	2.22	2.15	2.08
Min. (UID)	1.55	1.86	1.91	2.06	1.99	1.96
Max. (UID)	2.17	2.27	2.27	2.32	2.35	2.17
Mean (LID)	1.80	1.81	1.50	2.00	2.04	1.89
Min. (LID)	1.34	1.70	1.70	1.83	1.93	1.80
Max. (LID)	2.04	1.99	2.11	2.17	2.19	1.96
Mean (MID)	2.36	2.38	2.52	2.62	2.6	2.45
Min. (MID)	1.80	2.22	2.32	2.42	2.47	2.29
Max. (MID)	2.63	2.68	2.73	2.81	2.84	2.55
Mean (CHL)	0.14	0.15	0.13	0.15	0.16	0.14
Min. (CHL)	0.10	0.11	0.08	0.13	0.11	0.11
Max. (CHL)	0.16	0.20	0.29	0.16	0.20	0.15
Mean (TW)	2.61	2.54	2.82	2.8	2.73	2.64
Min. (TW)	2.25	2.19	2.58	2.53	2.47	2.53
Max. (TW)	2.89	2.99	3.20	2.99	2.99	2.99

group due to the absence of lateral pits on sternum 6 (Suppl. material 1: Appendix 1). In an additional 29 specimens, it was not possible to assign them to a species with certainty, due to their character combination (Suppl. material 6: Appendix 6). These aberrant specimens showed either a mix of species-specific characters of *C. succinctus* and *C. brevigena* or of *C. succinctus* and *C. hederae*. One specimen even showed characters of all three species. One specific male specimen from Velden in Carinthia (no. 124) could be assigned to the *C. succinctus* group due to its deep lateral pits on sternum 6. However, it differs from all other Austrian species by peculiar characters: the mesopleura are so densely punctured that the punctures merge into each other (as is only known from *C. halophilus*) and tergum 1 shows a very sparse puncturation with distances on disc about twice as long as a diameter of puncture. Tergum 2 is only superficially punctured.

Morphometric measurements (females)

Variation within species

Identified females of all species showed a pronounced intraspecific variation (Table 3). Especially, the head width (HW) seems to be very variable in all species. The intraspecific variation is most pronounced in *C. succinctus*. Here, the females differ very strongly in all measuring distances with the exception of the cheek length (CHL). *Colletes collaris* and *C. halophilus*, on the other hand, show a pronounced intraspecific variability in their head length (HL) and width (HW) and *C. brevigena* varies both in head width (HW) and thorax width (TW). *Colletes hederae* differ mostly in head width (HW) and upper interocular distance (UID). *Colletes pannonicus*, on the other hand, exhibits the lowest interspecific polymorphism, although the specimens differed to a large extent in their head width (HW).

On average, the females of *C. hederae* are the largest specimens (Table 3). They have by far the largest head, the longest clypeus, the longest lower interocular distance and the longest cheeks. In addition, they have the longest eyes, closely followed by *C. brevigena*. In turn, the largest average upper interocular distance is shown in females of *C. halophilus*, as well as the longest middle interocular distance, followed by *C. hederae*. The specimens of *C. brevigena* show the widest thorax on average, closely followed by *C. halophilus* and *C. hederae*.

Overall, the females of the species *C. succinctus* have the smallest mean head size (Table 2). They show the lowest values for head width, clypeus length, upper ocular distance, lower ocular distance and middle ocular distance. In terms of head length, they present the smallest mean value together with *C. pannonicus*.

Principal components analysis of all species (PCA)

All measurements were analysed using a principal component analysis (PCA). Based on 1,000 bootstrap re-samplings, the first principal component (PC1) explains just under 71% of the total sample variance, the second explains over 11% and the third explains about 7% of the variance.

The loadings of the nine measuring distances show that PC1 (principal component 1) correlates with all variables

Table 4. Loadings of principal component 1 (PC1), PC2 and PC3 for each measured distance of the six European members of the *Colletes succinctus* group. Measured distances: (HL) head length, (HW) head width, (EL) eye length, (CL) clypeus length, (UID) upper interocular distance, (LID) lower interocular distance, (MID) middle interocular distance, (CHL) cheek length, (TW) thorax width.

	PC1	PC2	PC3
HL (log)	0.313	0.071	-0.716
HW (log	0.375	-0.102	-0.140
EL (log)	0.359	-0.105	-0.345
CL (log)	0.348	0.170	0.082
UID (log)	0.360	0.001	0.334
LID (log)	0.378	-0.028	0.123
MID (log)	0.376	0.022	0.197
CHL (log)	0.074	0.941	0.090
TW (log)	0.303	-0.241	0.411

except for variable CHL (cheek length) (Table 4). Therefore, it is primarily a measure for body size. Principal component 2 dependents strongly on CHL. For example, when plotting PC1 and PC2, specimens of *C. hederae* are defined by high loadings on both axes, which means the majority have large bodies and long cheeks (Fig. 23). PC3 is strongly positively influenced by TW and negatively influenced by HL (Table 4). Thus, specimens with a wider thorax tend to have a shorter head.

Fig. 23 shows principal components 1 and 2, based on the measured values of the investigated species. Overall, the morphometric data of all species overlap with each other to some extent. By comparing only two species, it is possible to distinguish between some of them. The specimens of *C. hederae* and *C. succinctus* overlap only minimally. The same can be seen when comparing the data of *C. pannonicus* with *C. hederae* or *C. collaris*. However, measurements of all other species overlap greatly.

Table 5. Result of the linear discriminant analysis of the species *Colletes brevigena*, which is divided into the hypothetical groups *C. brevigena* MED (with Mediterranean origin) and *C. brevigena* A (Austrian specimens), including Jackknife re-sampling (1,000). Species in bold letters were classified differently from the hypothetical assignment.

Point	Given group	Classification	Jackknifed
42	*C. brevigena* MED	*C. brevigena* MED	***C. brevigena* A**
44	*C. brevigena* MED	*C. brevigena* MED	*C. brevigena* MED
45	*C. brevigena* MED	*C. brevigena* MED	***C. brevigena* A**
53	*C. brevigena* MED	*C. brevigena* MED	*C. brevigena* MED
70	*C. brevigena* MED	*C. brevigena* MED	*C. brevigena* MED
71	*C. brevigena* MED	*C. brevigena* MED	*C. brevigena* MED
74	*C. brevigena* MED	*C. brevigena* MED	***C. brevigena* A**
75	*C. brevigena* MED	*C. brevigena* MED	***C. brevigena* A**
76	*C. brevigena* MED	*C. brevigena* MED	*C. brevigena* MED
77	*C. brevigena* MED	*C. brevigena* MED	*C. brevigena* MED
78	*C. brevigena* MED	*C. brevigena* MED	*C. brevigena* MED
79	*C. brevigena* MED	*C. brevigena* MED	*C. brevigena* MED
161	*C. brevigena* MED	*C. brevigena* MED	***C. brevigena* A**
190	*C. brevigena* A	*C. brevigena* A	***C. brevigena* MED**
264	*C. brevigena* A	*C. brevigena* A	*C. brevigena* A
265	*C. brevigena* A	*C. brevigena* A	*C. brevigena* A
266	*C. brevigena* A	*C. brevigena* A	***C. brevigena* MED**
267	*C. brevigena* A	*C. brevigena* A	*C. brevigena* A

Linear discriminant analysis of all species (LDA)

Based on their morphological characters, the species could not be separated efficiently. Even with a Jackknife re-sampling, only 54.13% of all measured specimens were classified as their previously-assigned species (hypothetical group). Thus, around half of all specimens were assigned to different species by the LDA (Suppl. material 7: Appendix 7). A detailed listing of assignments for each measured specimen, by the author as well as by the LDA, with or without Jackknife re-sampling, is given in Suppl. material 8: Appendix 8.

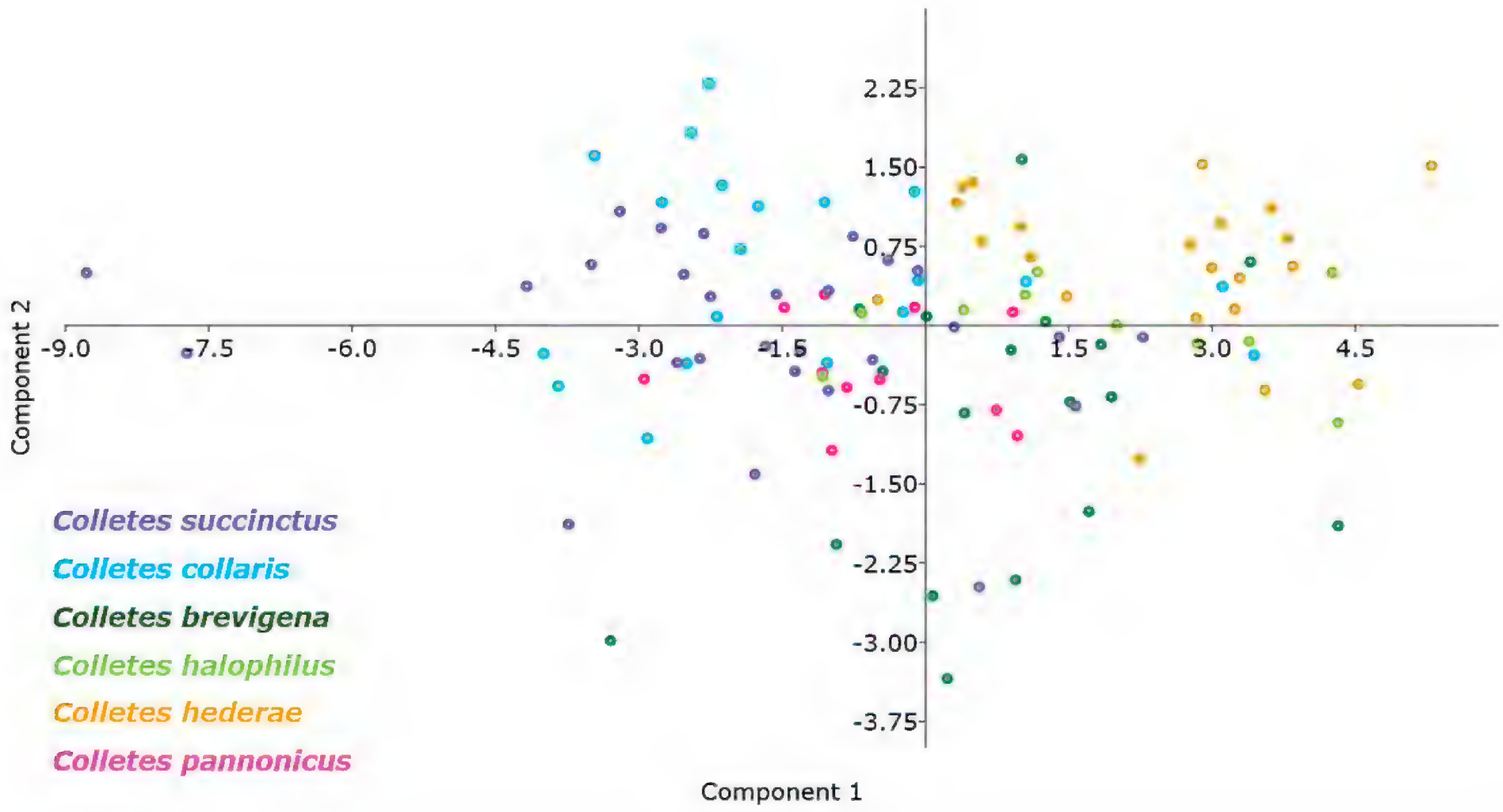

Figure 23. Scatter plot of PC 1 and PC 2, based on nine morphometric values of six European members of the *Colletes succinctus* group.

Linear discriminant analysis of *C. brevigena*

For a more detailed analysis of the species *C. brevigena*, all specimens (n = 18) were divided into two hypothetical groups: thirteen specimens from the Mediterranean region were grouped as "*C. brevigena* MED" and the remaining five females from Austria were grouped as "*C. brevigena* A". Based on the morphometric data, the discriminant analysis calculated that the specimens belonged to their previously-assigned hypothetical species in 100% of cases. Only after a Jackknife re-sampling, the affiliation of five Mediterranean *C. brevigena* and two Austrian *C. brevigena* was reversed, resulting in different assignments in about 40% (Table 5) of the specimens.

The taxonomically-challenging species *C. brevigena* and *C. pannonicus*

Head-thorax index: By comparing the head-thorax index of *C. brevigena* and *C. pannonicus*, the species cannot be differentiated. In relation to the thorax width, the examined females of *C. brevigena* show broader heads than the specimens of *C. pannonicus*; however, the two species overlap in their minimum to maximum head-thorax index range to a great extent (*C. brevigena* 71.7 to 87.5 vs. *C. pannonicus* 70.7 to 82.2).

Principal component analyses (PCA): Principal component 1 explains around 70% of the total sample variance and is defined by high positive loadings of all variables, except for variable CHL (cheek length) and TW (thorax width). Principal component 2, however, explains only 13% of the variance and shows high positive loadings of CHL and high negative loadings of TW.

Table 6. Loadings of principal component 1 (PC1) and PC2 for each measured character in members of *C. pannonicus* and *C. brevigena*. Measurements: (HL) head length, (HW) head width, (EL) eye length, (CL) clypeus length, (UID) upper interocular distance, (LID) lower interocular distance, (MID) middle interocular distance, (CHL) cheek length, (TW) thorax width.

	PC1	PC2
HL (log)	0.351	0.050
HW (log)	0.378	-0.123
EL (log)	0.366	-0.116
CL (log)	0.323	0.177
UID (log)	0.368	0.062
LID (log)	0.374	0.016
MID (log)	0.379	0.062
CHL (log)	0.100	0.837
TW (log)	0.255	-0.478

Therefore, PC1 is interpreted as a measure for body size, whereas PC2 is mainly a measure for sizes of CHL and TW (Table 6).

The examined specimens of *C. pannonicus* and *C. brevigena* overlap in their measurements to a great extent. On average, the specimens of *C. brevigena* are larger, but like in the PCA of all investigated species, the intraspecific variation is larger than the interspecific differences (Fig. 24).

Pollen analysis

Pollen determination

Based on their morphological characters, the pollen grains found on the studied specimens were assigned to

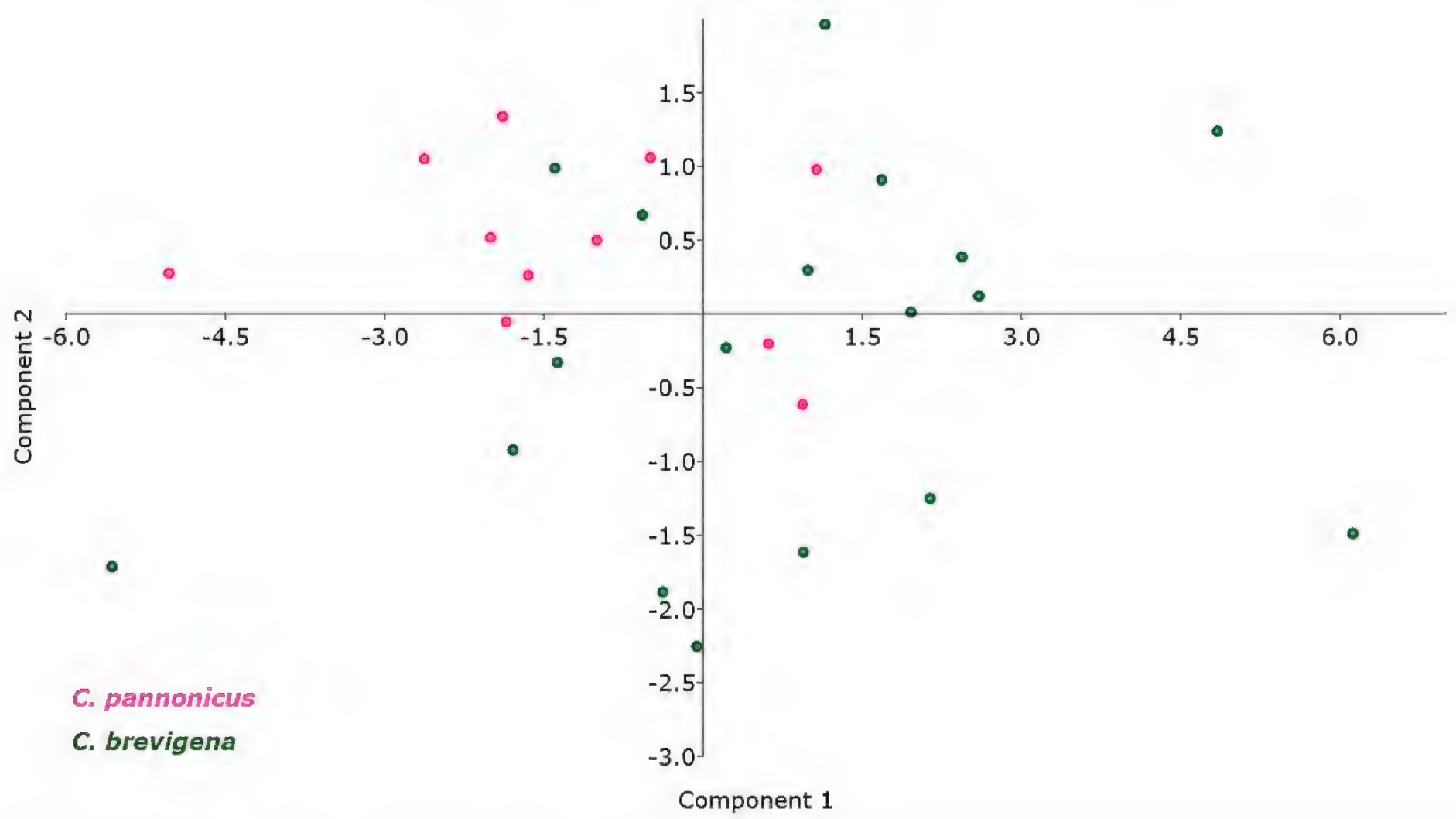

Figure 24. Scatter plot of PC 1 and PC 2, based on nine morphometric values of two European members of the *Colletes succinctus* group: (pink) *C. pannonicus*, (dark green) *C. brevigena*.

Asteraceae (liguliflorae and tubuliflorae), Araliaceae, Ericaceae, Resedaceae and Rutaceae (Fig. 25). In addition, tricolporate/reticulate pollen grains were also found in the pollen load of one specimen, but it was not possible to determine them more precisely. In some cases, it was possible to determine the pollen grains to genus level (*Citrus* sp., *Reseda* sp.) or even species level (*Tripolium pannonicum*, *Calluna vulgaris* and *Hedera helix*). The pollen of the family Asteraceae has a spiny (echinate) surface structure. They are either entirely spiked (tubuliflorae) or have spineless windows on their surface (liguliflorae). Ivy (*Hedera helix*) belongs to the Araliaceae family. Its pollen grains show a reticulate surface and are tricolporate: it has three colpi as well as three pores from which the pollen tubes emerge. Heather (*Calluna vulgaris*) belongs to the family Ericaceae and is arranged as tetradae: four spheroidal-shaped pollen grains are associated with each other. They possess pores and a scabrate to verrucate structure. The pollen of *Citrus* sp. (Rutaceae) is tetracolporate and with a reticulate surface.

Relationship between filling ratio and number of pollen types

Females with smaller pollen packages and therefore a lower filling ratio, collected fewer different pollen types than females with larger pollen loads and a high filling ratio ($r = 0.343$, $p = 0.003$).

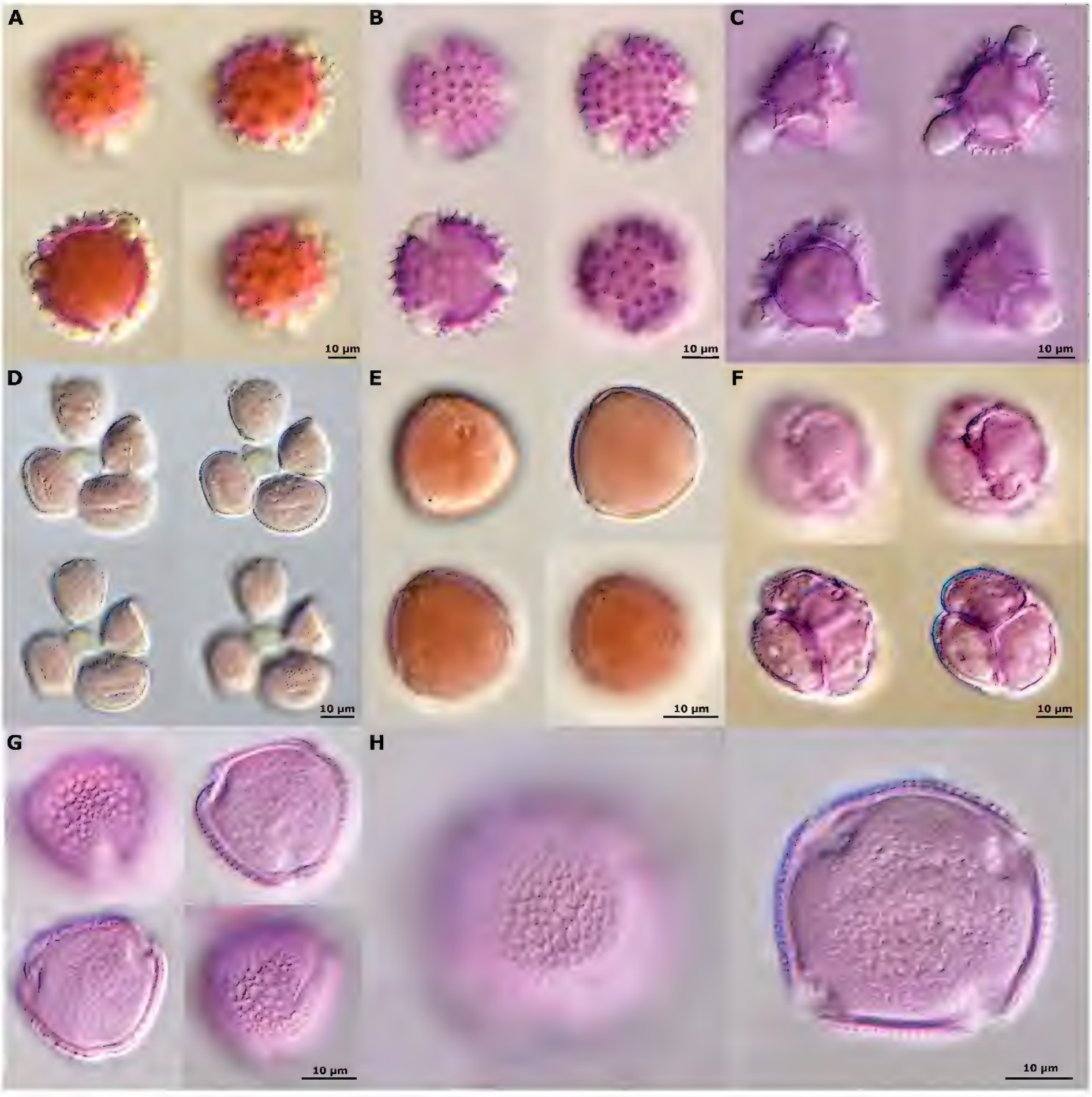

Figure 25. Selection of pollen grains collected by the females of the Austrian species of the *Colletes succinctus* group, scale bar 10 µm: **A.** Asteraceae tubuliflorae; **B.** Asteraceae tubuliflorae (*Tripolium pannonicum*); **C.** Asteraceae liguliflorae; **D.** tricolporate/reticulate; **E.** Resedaceae (*Reseda* sp.); **F.** Ericaceae (*Calluna vulgaris*); **G.** Araliaceae (*Hedera helix*); **H.** Rutaceae (*Citrus* sp.).

Pollen preferences of the Austrian species of the *C. succinctus* group

Colletes succinctus: Pollen analysis indicated that *C. succinctus* is polylectic. More than half (57%) of their average pollen load consisted of *Reseda* sp., closely followed by 40% Ericaceae pollen, which could be assigned to *Calluna vulgaris*. Only 1% of Asteraceae liguliflorae and 2% of Asteraceae tubuliflorae could be identified in the package and the rest was interpreted as contamination (Asteraceae, Ericaceae and undetermined). Fourteen of the 21 investigated *C. succinctus* females were collected in Retz, nine of them on *Calluna vulgaris* (Ericaceae) and five on *Reseda* sp. (Resedaceae): only seven of the nine specimens collected on *C. vulgaris* showed pure pollen packages consisting of that specific pollen. One female (no. 88) also preferred *C. vulgaris* (86.3%), but additionally collected a small proportion of Asteraceae liguliflorae (13.7%). Only in one specimen (no. 87), which was caught on *C. vulgaris*, no pollen of the same plant could be found. Its pollen package consisted of 100% *Reseda* sp. The five captured specimens on *Reseda* sp. possessed over 90% pollen from this plant in their collecting devices. One specimen (no. 92) additionally collected *C. vulgaris* in smaller quantities (8.3%) (Suppl. material 3: Appendix 3). Two *C. succinctus* females captured in Ollersdorf possessed pollen packages with over 99% *Reseda* sp. All specimens from Oberweiden (no. 160) and Bisamberg (n = 4) collected preferably on *Reseda* sp. Of those, only one female (no. 98) additionally collected 28.7% of its pollen package on Asteraceae tubuliflorae (Suppl. material 3: Appendix 3).

Colletes collaris: In summary, the 13 investigated females of *C. collaris* show a polylectic pollen-collecting behaviour with a strong preference for Asteraceae (66%). This is closely followed by *Reseda* sp. (31%). Only 2% are due to pollen of the type tricolporate/reticulate and 1% remained indeterminable. Flower consistency of specimens was rarely observed. A single female (no. 1), captured at Bisamberg, had a 100% pure pollen package of *Reseda* sp. and a further specimen (no. 3) also from Bisamberg collected 100% of Asteraceae of the type tubuliflorae. Resedaceae and Asteraceae tubuliflorae are also very popular with the remaining females from Bisamberg (nos. 4–7). One of them (no. 4) collected approximately equal parts of *Reseda* sp. (36.7%), Asteraceae tubuliflorae (33.3%) and tricolporate/reticulate pollen (30%). Another one (no. 6) preferred *Reseda* sp. (46%), closely followed by Asteraceae tubuliflorae (32%) and Asteraceae liguliflorae (16%) and a further 6% of the pollen load is attributed to contamination (Suppl. material 3: Appendix 3). A female (no. 9) from Langenlois preferred Asteraceae tubuliflorae (78.3%) to *Reseda* sp. (21.7%) as did the specimen from Engabrunner Haide (no. 10). The latter collected 76.7% of Asteraceae tubuliflorae and 23.3% of *Reseda* sp. (Suppl. material 3: Appendix 3). A female from Ollersdorf (no. 11) again preferred Asteraceae liguliflorae (56.7%) to Asteraceae tubuliflorae (43.3%). Another female from Ollersdorf (no. 195), caught on *Reseda* sp., collected mainly on this plant (77.3%), followed by Asteraceae tubuliflorae (21.3%). It shows a small amount of 1.3% that is presumably contamination-related (Suppl. material 3: Appendix 3). The three studied females from Lake Neusiedl (nos. 191–193) preferred Asteraceae tubuliflorae, which can be assigned to *Tripolium pannonicum*. Only no. 192 additionally collected 18.3% of its pollen load on Asteraceae liguliflorae (Suppl. material 3: Appendix 3).

Colletes hederae: *Colletes hederae* is the most represented species in this pollen analysis (n = 27). It shows a polylectic pollen-collecting behaviour with a strong preference for *Hedera helix* of the Araliaceae family (79.5%). In addition, 20.3% of the collected pollen comes from *Citrus* sp. of the family Rutaceae. There is only one specimen that shows contamination (0.2%) by *Citrus* sp. All females from Stammersdorf (no. 198), Hainburg (nos. 29–30), Linz (no. 221) and different parts of Vienna (nos. 221, 225 and 228–230) collected pure pollen packages of *H. helix* flowers. The specimens captured at Donaupark on *H. helix* (nos. 211–212 and 219) in turn possess not only pollen of *H. helix*, but also pollen of *Citrus* sp., some in smaller and some in larger quantities. One female (no. 217) collected 50% *H. helix* and 50% *Citrus* sp. (Suppl. material 3: Appendix 3).

Colletes brevigena: The only specimen of *C. brevigena* (no. 190) represented in this pollen analysis was caught at the same time as a specimen of *C. collaris* (no. 195) in Ollersdorf on the flowers of *Reseda* sp. This specimen collected *Reseda* pollen in large quantities (~ 98%) and only a small proportion of the pollen load is due to contamination (2.3%).

Colletes pannonicus: All specimens of *C. pannonicus* (n = 5) were captured near Lake Neusiedl and show an oligolectic behaviour, collecting pollen on Asteraceae tubuliflorae (99.6%), whereas only 0.4% of the load is due to contamination. Their individual pollen packages contain 98–100% pollen of this Asteraceae type, which can be assigned to *Tripolium pannonicum* and the contamination rate is ~ 2% (Suppl. material 3: Appendix 3).

Specimens without assignment to a species: Due to unclear morphological characters, seven specimens, which were used for pollen analyses, could not be clearly assigned to a specific species. Thus, they are marked with "???" in Suppl. material 3: Appendix 3. Most of these females were captured on the Bisamberg (n = 5) and chiefly collected *Reseda* sp. pollen. Two of them (nos. 62 and 64) additionally collected a small amount of pollen from Asteraceae tubuliflorae (13–19%). The other three (nos. 61, 63 and 65) had pollen loads with over 90% *Reseda* sp. and some amounts of contamination (0.3–2.0%). A female from Spitzerberg near Hainburg (no. 66) also preferred *Reseda* sp. (99.3%) and a single female (no. 148), for which both the date and location of capture are unknown, had a pure pollen package with *Calluna vulgaris* pollen (100%).

DNA-barcoding

Phylogeny

The obtained sequences were aligned for comparison (Suppl. material 9: Appendix 9). In the CO1 sequences of the specimens of *C. collaris*, 27 single base-pair differences were found, which clearly separate them from the other species. These, in turn, show little to no fixed substitutions in their alignment and therefore cannot be separate from each other.

The 21 obtained sequences, as well as 47 reference sequences received from the DNA-Barcode of Life Database (BOLD) (Suppl. material 5: Appendix 5), were used for further analysis by the neighbour-joining tree (NJT) method (Fig. 26). In the retrieved phylogenetic tree, the species *C. collaris* clusters in two clades strongly supported with Bootstrap values of 99% each and thus appears paraphyletic (as later found out, by a misidentification): clade 1 contains all the specimens from the present study (n = 4), as well as data from BOLD (n = 16), whereas clade 2 consists of two BOLD sequences (KC469653 and KC469654). The other four species (*C. brevigena*, *C. succinctus*, *C. hederae* and *C. pannonicus*) collapse into one large clade, which is supported by a moderate Bootstrap value of 88% (Fig. 26). They appear as the sister group to one specimen of *C. brevigena* (no. DQ085546), with a Bootstrap value of 99%.

For a better illustration of the relationships amongst these clustering species, a median joining network was created (Fig. 27). Most of the closely-related specimens share one common haplotype. As in the NJT, the two *C. collaris* clades are highly distinct. Clade 2 of *C. collaris* (KC469653 and KC469654), later identified as *C. luzhouensis* Kuhlmann, 2007, is separated from the main haplotype (*C. succinctus*, *C. brevigena*, *C. hederae* and *C. pannonicus*) by fifty substitution steps. One specimen of *C. succinctus* (no. BCZSM-HYM02017) and one specimen of *C. brevigena* (no. DQ085546) differ from the group by three substitution steps and two hypothetical haplotypes that could not be found in the sample.

Genetic distances

The interspecific distances between the investigated species are lowest between *C. brevigena* and *C. hederae* and highest between *C. collaris* and all the other species with the exception of the outgroup (*Colletes creticus*) (Table 7). The intraspecific distance, in turn, is slightly higher than the interspecific distance in *C. brevigena*, *C. succinctus*, *C. pannonicus* and *C. hederae*. It is the lowest amongst the *C. collaris* examined in this study (Table 8). Some of the investigated specimens of *C. brevigena*, *C. succinctus* and *C. hederae* have identical sequences and show

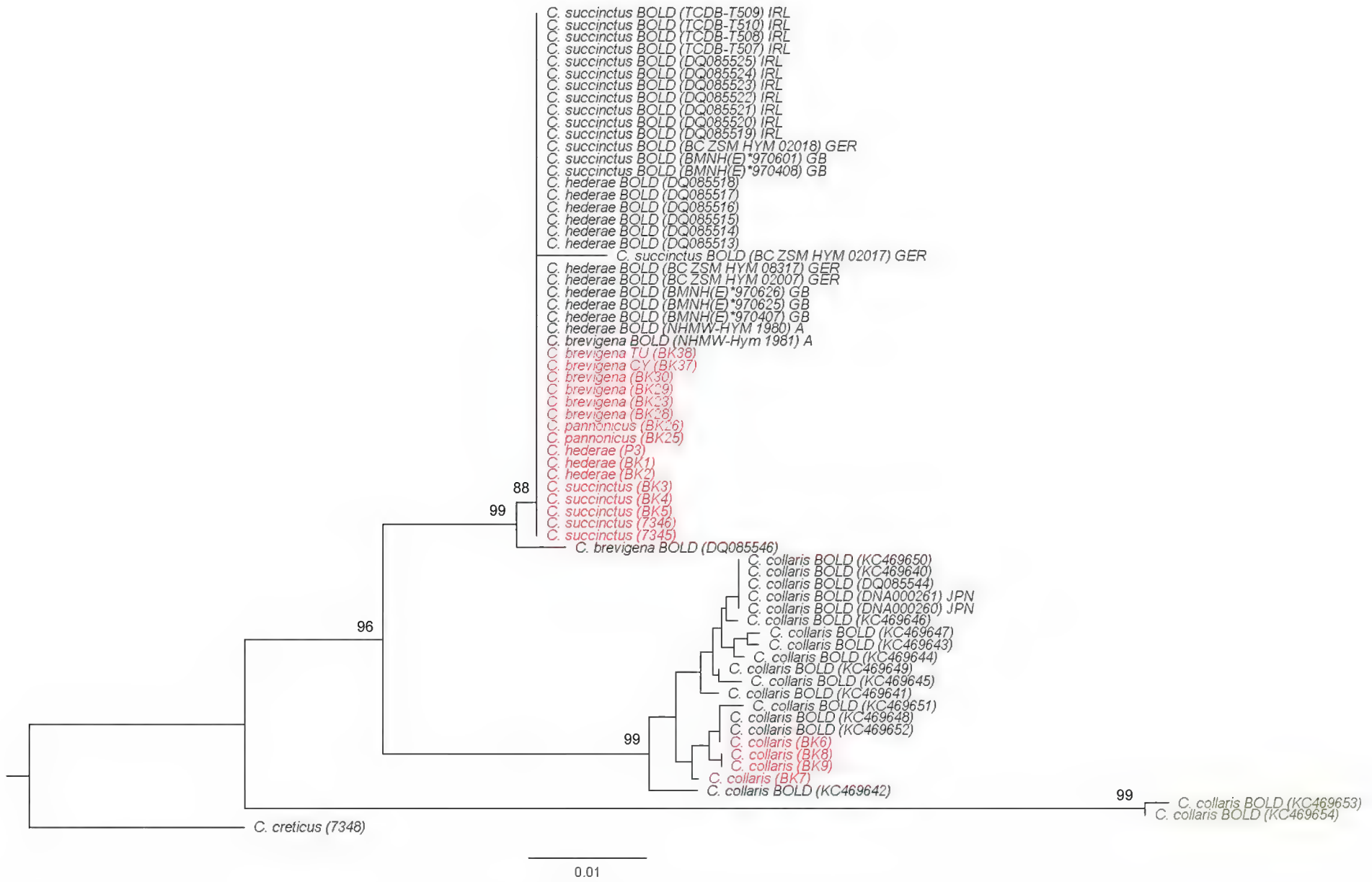
C. succinctus BOLD (TCDB-T509) IRL
C. succinctus BOLD (TCDB-T510) IRL
C. succinctus BOLD (TCDB-T508) IRL
C. succinctus BOLD (TCDB-T507) IRL
C. succinctus BOLD (DQ085525) IRL
C. succinctus BOLD (DQ085524) IRL
C. succinctus BOLD (DQ085523) IRL
C. succinctus BOLD (DQ085522) IRL
C. succinctus BOLD (DQ085521) IRL
C. succinctus BOLD (DQ085520) IRL
C. succinctus BOLD (DQ085519) IRL
C. succinctus BOLD (BC ZSM HYM 02018) GER
C. succinctus BOLD (BMNH(E)*970601) GB
C. succinctus BOLD (BMNH(E)*970408) GB
C. hederae BOLD (DQ085518)
C. hederae BOLD (DQ085517)
C. hederae BOLD (DQ085516)
C. hederae BOLD (DQ085515)
C. hederae BOLD (DQ085514)
C. hederae BOLD (DQ085513)
C. succinctus BOLD (BC ZSM HYM 02017) GER
C. hederae BOLD (BC ZSM HYM 08317) GER
C. hederae BOLD (BC ZSM HYM 02007) GER
C. hederae BOLD (BMNH(E)*970626) GB
C. hederae BOLD (BMNH(E)*970625) GB
C. hederae BOLD (BMNH(E)*970407) GB
C. hederae BOLD (NHMW-HYM 1980) A
C. brevigena BOLD (NHMW-Hym 1981) A
C. brevigena TU (BK38)
C. brevigena CY (BK37)
C. brevigena (BK30)
C. brevigena (BK29)
C. brevigena (BK33)
C. brevigena (BK28)
C. pannonicus (BK26)
C. pannonicus (BK25)
C. hederae (P3)
C. hederae (BK1)
C. hederae (BK2)
C. succinctus (BK3)
C. succinctus (BK4)
C. succinctus (BK5)
C. succinctus (7346)
C. succinctus (7345)
C. brevigena BOLD (DQ085546)
C. collaris BOLD (KC469650)
C. collaris BOLD (KC469640)
C. collaris BOLD (DQ085544)
C. collaris BOLD (DNA000261) JPN
C. collaris BOLD (DNA000260) JPN
C. collaris BOLD (KC469646)
C. collaris BOLD (KC469647)
C. collaris BOLD (KC469643)
C. collaris BOLD (KC469644)
C. collaris BOLD (KC469649)
C. collaris BOLD (KC469645)
C. collaris BOLD (KC469641)
C. collaris BOLD (KC469651)
C. collaris BOLD (KC469648)
C. collaris BOLD (KC469652)
C. collaris (BK6)
C. collaris (BK8)
C. collaris (BK9)
C. collaris (BK7)
C. collaris BOLD (KC469642)
C. collaris BOLD (KC469653)
C. collaris BOLD (KC469654)
C. creticus (7348)
88
99
96
99
99
0.01

Figure 26. Neighbour-joining tree of the sequences of the species of the *Colletes succinctus* group obtained in this study (n = 21, marked in red) and reference data (n = 47) from BOLD with outgroup and Bootstrap values (1,000 re-samples). The specimens KC469653 and KC469654 in BOLD (marked in green), originally assigned to *C. collaris*, were later identified as a different species, *C. luzhouensis* Kuhlmann, 2007, that does not belong to the *C. succinctus* group. The scale bar represents 0.01 substitutions per site.

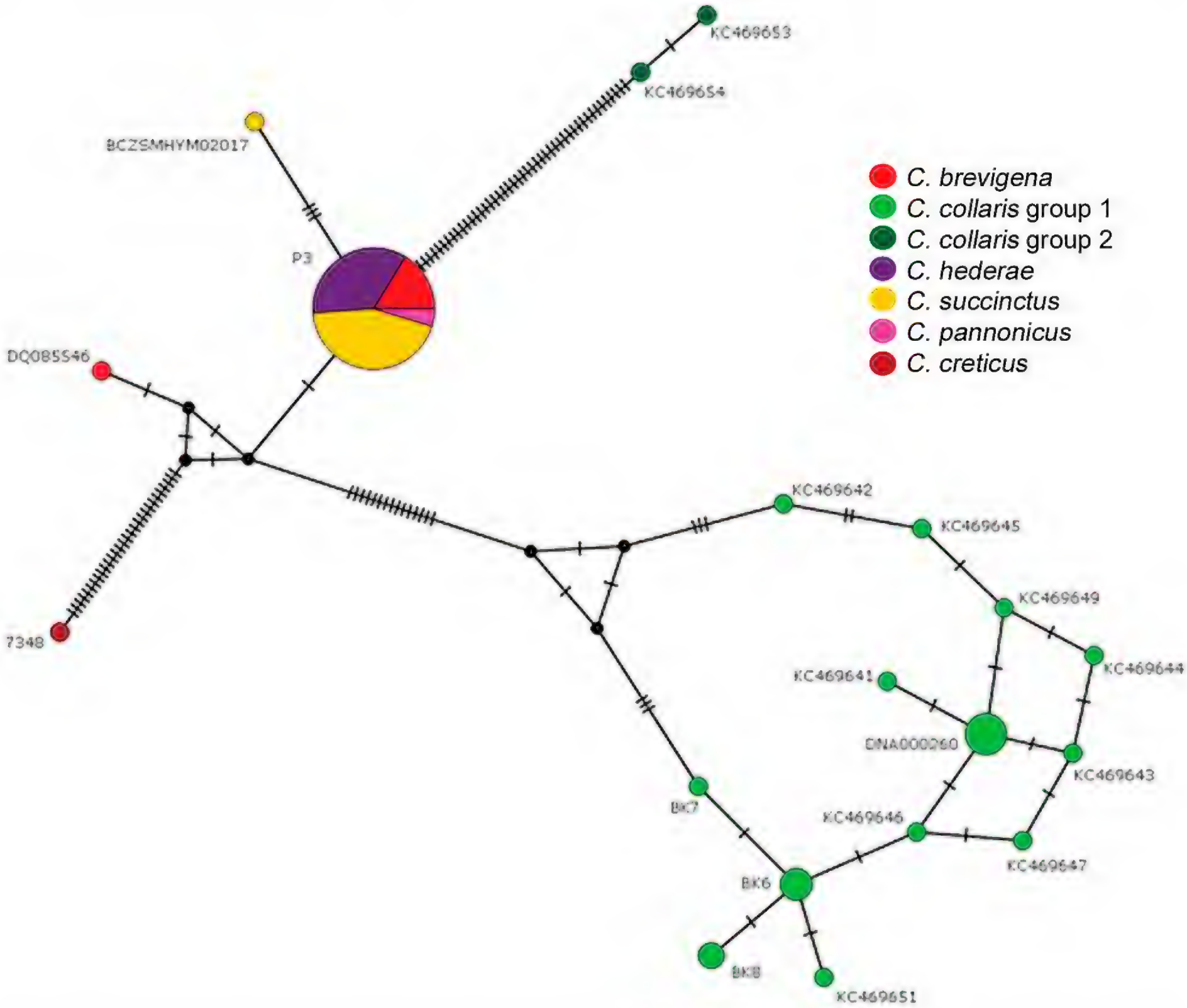

Figure 27. Median-joining network of the analysed species of the *Colletes succinctus* group (n = 20) and reference data (n = 47) from BOLD (Barcode of Life Database). (tick marks) substitution steps, (black dots) hypothetical haplotype that cannot be found.

no genetic distance (Table 7). A pairwise genetic distance matrix can be found in Suppl. material 10: Appendix 10.

Including sequence data from BOLD shows no differences. In addition, in this combined dataset, *C. collaris* is the most differentiated species (Tables 7 and 8). However, two sequences from BOLD, labelled as *C. collaris*, were highly differentiated resulting in a higher intraspecific distance than the interspecific distances to the other ingroup species and even higher than distances between ingroup and outgroup (Table 7).

Table 7. Genetic mean, minimum (min.) and maximum (max.) p distances (%) between the European species of the *Colletes succinctus* group, based on the specimens investigated (A) as well as on reference data (B) from the Barcode of Life Databank (BOLD).

Species 1	Species 2	Mean distance A	Mean distance B	Min. A	Min. B	Max. A	Max. B
C. hederae	*C. succinctus*	0.50	0.03	0.00	0.00	1.00	0.60
C. hederae	*C. collaris*	5.20	4.84	4.90	0.00	5.30	10.38
C. succinctus	*C. collaris*	5.10	4.87	4.90	3.99	5.40	10.38
C. hederae	*C. pannonicus*	0.50	0.00	0.30	3.99	0.80	0.00
C. succinctus	*C. pannonicus*	0.50	0.03	0.30	0.00	0.60	0.60
C. collaris	*C. pannonicus*	4.80	4.84	4.60	3.99	4.90	10.38
C. hederae	*C. brevigena*	0.40	0.07	0.00	0.00	1.00	0.60
C. succinctus	*C. brevigena*	0.50	0.10	0.00	0.00	1.00	0.60
C. collaris	*C. brevigena*	5.20	4.87	4.90	3.99	5.30	10.98
C. pannonicus	*C. brevigena*	0.50	0.07	0.20	0.00	0.80	0.60
C. hederae	*C. creticus*	6.80	6.19	6.80	6.19	6.80	6.19
C. succinctus	*C. creticus*	6.90	6.22	6.80	6.19	7.00	6.19
C. collaris	*C. creticus*	7.90	8.02	7.80	7.39	8.00	11.58
C. pannonicus	*C. creticus*	6.80	6.19	6.70	6.19	6.80	6.19
C. brevigena	*C. creticus*	6.80	6.19	6.70	6.19	6.80	6.19

Discussion

Morphology

New features were found for species differentiation in the Austrian *C. succinctus* group. Nevertheless, there is a pronounced intraspecific variation (between populations of different collecting sites) in all species. This also concerns previously-described diagnostic characters for females (Noskiewicz 1936; Schmidt and Westrich 1993; Burger 2010; Hölzler and Mazzucco 2011), like the wrinkles on the clypeus, the puncturation on the mesonotum

Table 8. Genetic mean p distance within European species of the *Colletes succinctus* group, based on investigated specimens (A), as well as merged with reference data (B) from Barcode of Life Database (BOLD).

	Mean distance A	Mean distance B
C. hederae	0.6	0.0
C. succinctus	0.5	0.1
C. collaris	0.2	2.5
C. pannonicus	0.5	0.0
C. brevigena	0.4	0.1

and the presence/absence of the reticulate micro-sculpture on the galea. Due to these sometimes very confusing character combinations, it was not possible to clearly assign all specimens of this study to a species. A few female specimens showed combined traits of the species *C. succinctus* and *C. hederae* or *C. succinctus* and *C. brevigena*, one even a mixture of characters of all three species. This can best be explained by the hypothesis that, in these young species, not all diagnostic characters are yet fixed in all populations.

By combining the new features with the already-known characters from literature (Noskiewicz 1936; Verhoeff 1944; Schmidt and Westrich 1993; Hölzler and Mazzucco 2011; Amiet et al. 2014), it was possible to establish identification keys for females from Austria and males from Central Europe. With these identification keys, the species *C. succinctus*, *C. collaris* and *C. hederae* can be reliably distinguished in both sexes. *Colletes halophilus* and *C. pannonicus* live in very similar habitats, but were never compared to each other. The initial hypothesis that they could be synonyms was not corroborated: the dorsobasal knob on the membrane of the gonostylus distinguishes *C. halophilus* from all other Central European species. *Colletes brevigena* and *C. pannonicus* remain to be difficult to determine as they are very similar in appearance. Only slight differences of the dorsal shape of the female's fovea facialis, as well as the male's genitalia, provide indications for the two-species hypothesis. The cuticle of the fovea facialis bears many secretory cells and is more strongly developed in females than in males (Schuberth and Schönitzer 1993). The differently shaped dorsal margins of the fovea are often used as a character for species identification in other genera, especially *Andrena* (Schmid-Egger and Scheuchl 1997).

Morphometry

The determination of females by use of the new identification key is not supported by the discriminant analysis (LDA). Based on their morphometric data, the LDA assigned only about half of all specimens to the same species as previously determined by the authors. For this method, specimens of *C. collaris* were used as reference species. *Colletes collaris* possesses several morphological characters (pilosity of propodeum, narrow band of setae on terga etc., compare Amiet et al. 2014) which do not show intraspecific polymorphism. As *C. collaris* was observed to have been mis-assigned by the discriminant analysis, the character sets, which were used for the LDA, are not suitable to differentiate the species.

In general, the morphometric analysis of the selected head characters and thorax width alone did not prove to be informative enough to distinguish females of the examined species of the *C. succinctus* group, as, for example, suggested for *C. pannonicus* by Hölzler and Mazzucco (2011). No morphometric studies have yet been published for the studied species, but other species of the genus *Colletes* have already been investigated: because of their similar appearance, especially in their puncturation on terga, *C. inexpectatus* Noskiewicz, 1936 and *C. daviesanus* Smith, 1846 were regarded as synonyms (Warncke 1978). Přídal (1999) was able to verify that both represent independent species, amongst other characters, by the measurements of the male's hind tarsus. Therefore, the present results call for subsequent examinations. Maybe it is possible to gain more information about species differentiation by measuring legs or antennae. Additionally, males should be added to morphometric studies.

Pollen Analysis

In this study, the species of the *Colletes succinctus* group occurring sympatrically in Austria were both polylectic and oligolectic: the investigated females of *C. succinctus*, *C. collaris* and *C. hederae* showed a polylectic pollen-collecting behaviour, *C. pannonicus* appeared to be oligolectic, but examination was based on a single population. In addition, a correlation between the filling ratio of the pollen packages and the number of different pollen types could be determined: the larger the pollen load, the more different pollen types could be found.

The present study showed a preference of the "heather bee" *C. succinctus* for *Reseda* sp. (hitherto unknown as a pollen source), closely followed by *Calluna vulgaris* and Asteraceae, whereas Müller and Kuhlmann (2008) found that the species collected pollen on Ericaceae, Araliaceae, Asteraceae and Apiaceae. Interestingly, some specimens of the *C. succinctus*, collected in Retz, had the expected pollen of *Calluna vulgaris*, but some others used the pollen of *Reseda* sp. thriving in close vicinity, although there was no obvious shortage of *Calluna* flowers. That the females had either pure *Calluna vulgaris* or *Reseda* sp. pollen loads can be explained by flower consistency (cf. Waser 1986).

The single analysed female of *C. brevigena* had a pollen load of pure *Reseda* sp.; this species is described as polylectic by Müller and Kuhlmann (2008), collecting pollen on a variety of different families. These authors also described *C. hederae* as polylectic, whereas Bischoff et al. (2005) described the species as oligolectic due to the fact that examined nest cells and pollen loads only showed pollen of *Hedera helix*. In this study, most of the females collected pure pollen loads from *Hedera helix* flowers. Only females from Donaupark in Vienna showed polylectic behaviour by adding pollen of *Citrus* sp. to

their pollen packages. This plant genus is recognised for the first time as a pollen source for *C. hederae*. The other females of *C. hederae* were captured in Linz and several sites in Vienna, directly on *H. helix*. During the rather late flight period of *C. hederae* (September–November; Kuhlmann et al. 2009), there is no particularly large selection of flowering plants available. Ivy flowers offer an easily accessible and nutrient-rich pollen resource. Nonetheless, it was observed that, if other nutritious pollen sources are available, *C. hederae* disregards *Hedera* sp. and starts collecting pollen from the other sources. Therefore, the polylectic pollen-collecting behaviour of *C. hederae* is sometimes referred to as pseudo-oligolectic; this means that they are sometimes only oligolectic by lack of choice (Teppner and Brosch 2015).

Little is known about the pollen-collecting behaviour of *C. pannonicus*. Field observations led to the assumption that *C. pannonicus* is strictly oligolectic on *Tripolium pannonicum* (sea aster) (Hölzler and Mazzucco 2011). The first pollen analysis, conducted within this study, confirmed that *C. pannonicus* from the same population is oligolectic on Asteraceae. However, as all investigated females were collected on *T. pannonicum*, the results can either be explained by strict oligolectic behaviour or by flower consistency.

Although the examined females of *C. collaris* showed a strong preference for Asteraceae, which is in accordance with findings by Müller and Kuhlmann (2008), they also collected pollen of *Reseda* sp. Asteraceae are omnipresent, provide large amounts of pollen and nectar and flower, depending on the species, from spring to autumn, but they have a low protein and amino acid content (Somerville and Nicol 2006) and a possibly toxic pollen kit (Williams 2003) and the extraction of important nutrients from the pollen plasma is difficult (Peng et al. 1985). Therefore, expensive physiological adaptations of the bees to this pollen type are necessary: for coping with a low protein and amino acid content, for detoxification and for an easier extraction of important nutrients from the pollen plasma (Müller and Kuhlmann 2008). Asteraceae are the perfect nutrient supplier for adapted bees, being available almost without competition. In *Colletes*, Asteraceae are preferentially collected by oligolectic species, but are largely avoided by the majority of polylectic species; exceptions are foremost found of the *Colletes succinctus* group: *C. succinctus*, *C. brevigena* and *C. hederae* (Müller and Kuhlmann 2008).

DNA-barcoding

Only specimens of *C. collaris* can be clearly separated from the other species. By analysing only sequences of the material of this study, *C. collaris* forms a monophylum and the sister group to the other analysed species, which collapse into one large clade and show little to no genetic distance to each other. However, after adding sequences from BOLD, *C. collaris* separates into two clades and forms a paraphylum. This can be explained by checking the two *C. collaris* sequences that appear secluded in the tree: an additional BOLD blast showed that both specimens belong to *Colletes luzhouensis* Kuhlmann, 2007, a species native to China, which explains not only the high intraspecies genetic distance of *C. collaris*, but also the high genetic distances to the other species. Thus, *C. collaris* forms a monophylum and is the sister group to a clade comprising the remaining studied species of the *C. succinctus* group. A previous study of Kuhlmann et al. (2009), albeit including only one specimen of *C. collaris*, analysed the gene fragments *CO1* and *28S* and achieved the same result: *C. collaris* represented the sister group to *C. intricans*, *C. succinctus*, *C. hederae*, *C. brevigena* and *C. halophilus*.

In this study, *C. pannonicus* was examined for the first time by using DNA barcoding. However, it cannot be distinguished from the other investigated species, except *C. collaris*. Since the species collapse into one large clade/haplogroup (except *C. collaris*), it is not possible to assign specimens to species using the *CO1* sequence, which is in accordance with previous studies (Kuhlmann et al. 2007, 2009). For future investigations of the group, it would be recommended to investigate other genes.

The challenging species *Colletes brevigena* and *C. pannonicus*

Colletes brevigena and *C. pannonicus* proved to be the most challenging species. Due to their similar appearance, it is difficult to distinguish them strictly by morphology. There is a small difference in the female's fovea facialis that was not mentioned in the original description of the holotype of *C. pannonicus* (Hölzler & Mazzucco, 2011), whereas the previously-stated morphometric differences could not be approved in larger material. The previously undescribed male of *C. pannonicus* shows only discrete differences in its genitalia. Furthermore, some Austrian specimens of *C. brevigena* showed different morphological character states than specimens from the Mediterranean. Therefore, both species were investigated more closely.

All examined specimens of *C. pannonicus* were found solely near to the type locality in the Seewinkel where they can be observed flying around the flowers of *Tripolium pannonicum* (sea aster). That all known specimens were caught around Lake Neusiedl is surprising, as there are no geographical barriers which would prevent a wider distribution. Specialisation to a distinct habitat, salt meadows, seems the most likely and hitherto accepted explanation that *C. pannonicus* could not be found elsewhere in Austria. The proposed (Hölzler and Mazzucco 2011) and here confirmed apparent dependence on *T. pannonicum* could be explained by the fact that hardly any other Asteraceae are blooming at these sites in late autumn. *Tripolium pannonicum* is a widespread plant found in Europe and in temperate regions of Asia (Euro+Med 2006) and it can be

expected that *C. pannonicus* has a wider distribution than presently known. The seeming endemism is either caused by under-collecting in other suitable areas or, possibly, by confusion with similar species, mainly *C. brevigena*.

Noskiewicz (1936) described *C. succinctus* ssp. *brevigena*, based on specimens from a large distribution area spanning from the Balkans, to Crete, Persia and the Caucasus, but many specimens are untraceable. The selection of a lectotype was necessary to define this problematic species. The type locality is Crete (Greece).

A clear morphological distinction could be detected between Austrian specimens (from Bisamberg and Ollersdorf, Lower Austria) of *C. brevigena* and specimens from the Mediterranean region. The Austrian females are unusually large and show a puncturation on terga, mesonotum and mesopleura that is similar to *C. succinctus* and/or *C. hederae*. Only some specimens from Spitzerberg (Lower Austria) are more similar to Mediterranean *C. brevigena*. A linear discriminant analysis (LDA) of morphometric data resulted in clear separation of Austrian and Mediterranean specimens (100%). Subsequently, in a Jackknife re-sampling, only 60% of all specimens could be assigned to their original group (Austrian or Mediterranean). This lower value may be explained by the small number of Austrian specimens (n = 5) in comparison to the higher number of Mediterranean specimens (n = 13). To determine whether Austrian and Mediterranean specimens differ in their measurements or are more similar than assumed in this study, a larger number of samples would be needed for measurement. Unfortunately, due to the rarity of this species in Austria, this was not possible during this study.

Zettel et al. (2006) also listed questionable females of *C. brevigena* in Ollersdorf. These specimens showed a puncturation on their terga that is similar to the puncturation of *C. hederae*, but as typical for females of *C. brevigena*, they have longitudinal wrinkles on their clypeus. Therefore, the authors classified them with reservation as *C. brevigena* (Zettel et al. 2006).

Concluding the findings for *C. brevigena*, this study raises doubts about the close relationship between specimens/populations of *C. brevigena* occurring in Austria and specimens from the Mediterranean region. In addition to the morphological differences, also the phenology differs: in the Mediterranean, *C. brevigena* is bivoltine (Kuhlmann 2003; Standfuss 2009): the first generation flies in May and the second generation later in the year (according to collection data from September to November). In Austria, however, there is only one generation active from August to September (Zettel et al. 2006). It can be assumed that *C. brevigena* is a Mediterranean species that post-glacially migrated northwards, where it must have adapted to different environmental conditions, most importantly to a shorter warm season. In this case, it would be expected that – like in some other bee species (e.g. *Andrena pontica* Warncke, 1972; Scheuchl and Willner 2016) – the second generation is omitted because of the longer-lasting development. In the peculiar case of *C. brevigena*, however, the spring generation is omitted. As most specimens of the genus *Colletes* in Central Europe spend their diapause (hibernation) as a pre-pupa (in the last larval stage) (Westrich 1989), this delayed development into an imago should be genetically fixed and, therefore, must be a trait that was passed on by its ancestor. A similar case is also known from the species complex of *Andrena argentata* Smith, 1844. This bivoltine species shows a trans-Palaearctic distribution. In England and Sweden, it either has no spring generation or the summer generation is richer in specimens. Additionally, in this case, it is assumed that the two generations do not belong to the same species (Scheuchl and Willner 2016).

Thus, it would be quite possible that the Austrian specimens in question are of a different species that is very similar to *C. brevigena* in the Mediterranean. However, it is also possible that *C. brevigena* shows a highly pronounced geographic variation. The studied populations in Austria and the Mediterranean region are geographically far apart and differ to such an extent that they can be regarded as conspecific only with difficulty. It would be advisable to study *C. brevigena* populations from intermediate areas, for example, from Hungary or the northern Balkans. Thereby, it may be possible to find transitional morphs that could corroborate the conspecificity of the two morphologically different groups. Both possibilities would merit further investigation.

Integrative approach to separate *C. brevigena* and *C. pannonicus*

Colletes brevigena and *C. pannonicus* share a very similar morphological character set. Genetic data (mitochondrial gene *CO1*) are not useful to differentiate between them. It is difficult to distinguish the two species by the discriminating characters described by Hölzler and Mazzucco (2011), as morphometric data greatly overlap. For example, Hölzler and Mazzucco (2011) stated that the female of *C. pannonicus* possesses a wider head in relation to the thorax width, when compared to *C. brevigena*. This was not confirmed by this study. The values of both species greatly overlap, with *C. brevigena* even showing mean values for a slightly wider head than *C. pannonicus*. These findings can support the assumption from the previous chapter, that *C. pannonicus* can easily be misidentified as *C. brevigena*. In the salt meadows of Seewinkel, however, the two species do not occur sympatrically, but *C. pannonicus* shares the habitat with *C. collaris*. *Colletes brevigena*, on the other hand, is only found in steppe biotopes (Zettel et al. 2006). Due to the few subtle differences, it is quite possible that specimens of *C. pannonicus* were misidentified as *C. brevigena* in other areas of south-eastern Europe. Additionally, the oligolectic collecting behaviour on *Tripolium pannonicum* in Seewinkel could simply be based on the lack of alternative resources. Therefore, it is important to include pollen preferences, as well as habitat preferences, to differentiate between both species.

Specimens without assignment to a species

For 21 specimens, it was not possible to assign them to a species, based on their morphological characters alone. They showed mixed characters of several species (*C. succinctus*, *C. brevigena* and *C. hederae*), especially, regarding the puncturation on terga, mesopleura and mesonotum, as well as structures on clypeus and galea.

The intraspecific variation of the species of the *C. succinctus* group has always been an issue for taxonomists (Noskiewicz 1936; Verhoeff 1944; Schmidt and Westrich 1993; Hölzler and Mazzucco 2011). Some specimens show mixed characters and cannot be clearly identified. Therefore, the ecology (pollen preferences of females and phenology) has been included in species differentiation (Kuhlmann et al. 2007). For example, female no. 148 shows *C. succinctus*-typical terga, mesopleura and clypeus, but does not have a shiny centre on the mesonotum. Since this was already mentioned by Noskiewicz (1936) as a typical *C. succinctus* characteristic, further aspects have to be investigated. When examining the pollen package of the specimen, it consists purely of *Calluna vulgaris*. Thus, the probability is very high that this specimen is a specimen of *C. succinctus* which, however, did not develop all characters typical for this taxon. In addition, the females (nos. 61–66), which were collected at Bisamberg (Vienna), show combined characters of *C. succinctus*, *C. brevigena* and *C. hederae*. These specimens were collected mid-September on *Reseda* sp. (pollen loads studied). Although *Reseda* sp. is only known as a pollen source for *C. brevigena* and *C. collaris* in literature (Müller and Kuhlmann 2008) and for *C. succinctus* in the present study, it is not impossible that *C. hederae* also collects pollen on this flower. *Colletes hederae* is a polylectic species, which shows preferences for *Hedera helix*, but also collects pollen on other plants, should their preferred plant not yet be in bloom (Müller and Kuhlmann 2008; Westrich 2008; Teppner and Brosch 2015). Moreover, flower visits of *C. hederae* on *Reseda* sp. have been observed after this study was carried out (H. Zettel, unpubl.).

Conclusion

Based on the results presented here, it can be assumed that the species of the *C. succinctus* group are either species *in statu nascendi* or evolutionary of very recent origin. In any case, incomplete lineage sorting, as well as gene flow, might explain the close genetic relationships. This study was able to find further helpful characters for a morphological identification of the Austrian species of the *C. succinctus* group. The main result is that the species complex *C. succinctus-brevigena-hederae-pannonicus* is more complicated than assumed by all previous taxonomists (Noskiewicz 1936, Verhoeff 1944, Schmidt and Westrich 1993, Hölzler and Mazzucco 2011), as there is high variation in morphological and ecological characters. For some specimens, it is still difficult to identify them by studying their morphology. Thus, the ecology of the specimens continues to be an important tool for species differentiation.

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Supplementary material 1

Appendix 1

Authors: Katharina Zenz, Herbert Zettel, Michael Kuhlmann, Harald W. Krenn

Data type: Specimen data

Explanation note: Examined specimens of the Austrian species of the *Colletes succinctus* group, sorted by ID number, including genus, species (currently assigned and previously assigned), sex, collecting site and date, owner of specimen as well as all applied approaches.

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Link: https://doi.org/10.3897/dez.68.55732.suppl1

Supplementary material 2

Appendix 2

Authors: Katharina Zenz, Herbert Zettel, Michael Kuhlmann, Harald W. Krenn

Data type: Morphometrical data

Explanation note: List of all morphometrically measured individuals, listed by ID number, including species and measured values in millimetres.

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Supplementary material 3

Appendix 3

Authors: Katharina Zenz, Herbert Zettel, Michael Kuhlmann, Harald W. Krenn

Data type: Specimen data

Explanation note: List of all individuals of the Austrian species of the *Colletes succinctus* group used for pollen analysis, listed by species.

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Supplementary material 4

Appendix 4

Authors: Katharina Zenz, Herbert Zettel, Michael Kuhlmann, Harald W. Krenn

Data type: Specimen data

Explanation note: List of specimens used for DNA barcoding, including ID number, genus, species, sex, sequencing number and status of sequencing.

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Link: https://doi.org/10.3897/dez.68.55732.suppl4

Supplementary material 5

Appendix 5

Authors: Katharina Zenz, Herbert Zettel, Michael Kuhlmann, Harald W. Krenn
Data type: *CO1* sequences
Explanation note: List of *CO1* sequences of specimens of the *Colletes succinctus* group, which were obtained from the Barcode of Life Database (BOLD). Including sample ID number, genus, species as well as sex and origin (if specified in database).
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Link: https://doi.org/10.3897/dez.68.55732.suppl5

Supplementary material 6

Appendix 6

Authors: Katharina Zenz, Herbert Zettel, Michael Kuhlmann, Harald W. Krenn
Data type: List of individuals
Explanation note: List of individuals for which no exact species affiliation could be determined.
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Link: https://doi.org/10.3897/dez.68.55732.suppl6

Supplementary material 7

Appendix 7

Authors: Katharina Zenz, Herbert Zettel, Michael Kuhlmann, Harald W. Krenn
Data type: Species assignment
Explanation note: Species assignment before and after the LDA, including Jackknife resampling (1,000).
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Link: https://doi.org/10.3897/dez.68.55732.suppl7

Supplementary material 8

Appendix 8

Authors: Katharina Zenz, Herbert Zettel, Michael Kuhlmann, Harald W. Krenn
Data type: Species assignment
Explanation note: Species assignment before and after the LDA, including Jackknife resampling (1,000). Species in bold letters were classified differently than the hypothetical assignment.
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Link: https://doi.org/10.3897/dez.68.55732.suppl8

Supplementary material 9

Appendix 9

Authors: Katharina Zenz, Herbert Zettel, Michael Kuhlmann, Harald W. Krenn
Data type: *CO1* sequences
Explanation note: Alignment of the self-obtained *CO1* sequences showing distinguishing substitutions of the Austrian species of the *Colletes succinctus* group and outgroup taxa (*C. creticus*).
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Link: https://doi.org/10.3897/dez.68.55732.suppl9

Supplementary material 10

Appendix 10

Authors: Katharina Zenz, Herbert Zettel, Michael Kuhlmann, Harald W. Krenn

Data type: Pairwise genetic mean p distances

Explanation note: Pairwise genetic mean p distances (%) between the examined individuals of the European species of the *Colletes succinctus* group, based on the individuals investigated.

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